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MANAGEMENT OF CHILDREN WITH INTERSEX CONDITIONS: PSYCHOLOGICAL AND METHODOLOGICAL PERSPECTIVES

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Pediatric medicine has undergone considerable upheaval in the past few years over the treatment of children with disorders of sexual differentiation. There have been challenges to all aspects of traditional practice, including sex assignment, genital surgery, the role of the patient and parents in decision-making, disclosure of medical details, the composition of the treatment team, and nomenclature. These challenges have been met with serious attention by pediatricians and other health professionals involved in the care of these children, and there has been considerable discussion of the merits of changes to current practice.[1-6] This report considers the status of the evidence relevant to treating children with intersex conditions, with particular emphasis on psychological and methodological issues.

BACKGROUND

For 50 years, treatment of children with intersex conditions was guided by the belief that gender identity results from social rearing rather than biological factors, provided that gender-confirming genital surgery is done early in life.[9,10] Although there have always been questions about this policy, anecdotal evidence generally suggested that it produced good outcome.[11,12] The policy and the evidence used to support it have recently been subject to detailed scrutiny because of several well-publicized reports. This includes a case of ablatio penis raised female who was unhappy with the assigned sex,[13,14] conference reports of XY males with absent or malformed penis due to cloacal exstrophy reared as females who declare themselves to be boys,[15] and reports of adverse outcomes from intersex patients.[16,17]

Several issues have emerged from recent discussions (Table 1). The focus has been on sex assignment and genital surgery, with traditional treatment and challenges often seen in polar terms (Table 2). Discussions have often been acrimonious, and recommendations based on personal beliefs or anecdotes, although it is clear that the interests of patients are best served by careful application of evidence.

EVIDENCE REGARDING SEX ASSIGNMENT

Determinants of Gender Identity

Decisions regarding sex assignment require recognition of the complexity of gender identity. Gender identity cannot be simply predicted from any single factor; neither is it always consistent with sex of rearing, nor is it simply related to extent of prenatal hormone exposure. The publicized individual with ablatio penis[14] was reared as a boy early in life and it is unclear how this contributed to his gender identity. Another individual with a similar history but with earlier female reassignment had a different outcome, particularly female gender identity.[18] To date, there have been no published systematic studies of individuals with cloacal exstrophy, and case reports indicate variations in gender identity, with no clear indication of the percentage who identify as males or are unhappy as females.[19,20]

The most systematic evidence regarding gender identity comes from two conditions. Females with congenital adrenal hyperplasia (CAH) overwhelmingly identify as female.[21-23] The very small minority of females with CAH who are unhappy as females or live as males are not necessarily those with the greatest genital virilization or

Highlights In This Issue

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Table 1

Controversies in Treatment of Children with Intersex Conditions

Sex assignment
What criteria should be used?
What determines gender identity?
When (if ever) is gender identity fixed?

Genital reconstructive surgery
Is it necessary? (Why?)
When should it be done?
What are its benefits and risks?

Decision-making
Who makes the decisions?
When should decisions be made?
What information is used to make the decisions?
What support is available?

Information-sharing
What are the parents told at the time of diagnosis and decision-making?
What does the child learn and when?
What support is available?
What is the best way to share information?

Involvement of mental health professionals
Should psychologists or psychiatrists be part of the diagnosis and treatment team?
Does counseling to families facilitate decision-making?
Does routine and continuing counseling to patients and families improve outcome?

the most prenatal androgen excess. Males with micropenis have not been studied as extensively as females with CAH, but they identify as males when reared that way and appear to function well.[24,25]

There is little systematic evidence to guide decisions about sex assignment in other intersex conditions.[20] Recent studies of individuals with micropenis and those with ambiguous genitalia with perineoscrotal hypospadias of varying etiology suggest that gender identity is generally consistent with sex of rearing.[26,27] But, for several reasons, caution is necessary when generalizing from these studies. First, a substantial proportion of participants (about 25%) were dissatisfied or questioned their sex of rearing. Second, as is typical of retrospective studies, patients who were dissatisfied or atypical were probably underrepresented: 30% of eligible patients did not participate and some participants elected not to answer sensitive questions. Third, outcome was assessed with a few items of unknown sensitivity. Fourth, those reared as boys were subjected to more surgery than those reared as girls.

Recommendations Regarding Sex Assignment

Sex assignment for an intersex child is one of the most difficult decisions made by parents and health professionals, though it is natural to seek simple solutions. But just as it is no longer tenable to assume that gender identity is always consistent with the sex of rearing, evidence indicates that it is equally unwise to consider gender identity to result directly from fetal androgen exposure (inferred from genital appearance or another indicator). Although other aspects of behavior may relate to degree of fetal androgen exposure, gender identity does not. For example, among females with CAH, degree of prenatal androgen exposure (inferred from genetic mutation, salt-wasting status, and degree of genital virilization) is moderately associated with interest in boy-typical activities and sexual orientation.[23,28-30] but not gender identity.[21-23] Therefore, it is crucial to separate aspects of outcome (Table 3).

There is sufficient evidence to suggest that 46,XX CAH patients be reared as girls, given the documented good outcomes associated with such rearing. Nevertheless, there are no systematic studies of those reared as boys. It is reasonable to suggest that 46,XY micropenis patients be reared as boys, given the small studies of good outcomes in such cases and the need for surgery with rearing as girls, but it would be helpful to have more evidence comparing quality of life and sexual function in those reared as boys vs. girls. In all other cases, decisions will need to be made with the limited information available from case reports. All children should be assigned as boys or girls. Rearing children as intersex is not advocated by health professionals or activist organizations (including ISNA). Parents and health professionals should realize that an intersex individual may elect to change gender later in life. The accuracy of the sex assignment can only be judged by the patient. It is essential to recognize that gender identity is not synonymous with gender-role behavior or sexual orientation, so that childhood tomboy behavior in girls or homosexuality should not be taken as indications of incorrect sex assignment.

EVIDENCE REGARDING SURGERY

Decisions regarding genitoplasty should be considered in light of the evidence regarding the stated need for surgery. Current practice is predicated on several assumptions: (1) sex-typical genital appearance is necessary for gender identity development consistent with rearing sex and for healthy psychological adjustment; (2) adjustment is hindered by unusual-appearing genitalia, through disruption in parent-child bonding, reactions from caretakers and peers, and difficulty in forming sexual relationships; (3) corrected genitalia are necessary for sexual activity, particularly intercourse. But some intersex patients as adults have

Table 2

Summary of Traditional Care and Current Challenges in the Treatment of Children with Intersex Conditions

Sex Assignment/Gender Identity	**Traditional Practice**	**Challenge**
Determinant of gender identity	sex of rearing	prenatal androgen
Stability of gender identity	fixed by age 2	develops throughout life
Role of genitalia	crucial to identity & adjustment	reflect brain masculinization
Decision-maker	physician	family
Genital Surgery		
Rationale	anatomy to match rearing sex	surgery is for comfort of others
Consequences	facilitates gender identity	inhibits gender change
	facilitates adjustment	impairs sexual function
	facilitates sexual intercourse	
Decision-maker	physician	patient

Table 3

Aspects of Outcome in Children with Intersex Conditions

Gender Identity	Sense of self as male or female
Gender-role Behavior	Aspects of behavior that differ between males and females; is multidimensional
Sexual Orientation	Sex of target of sexual arousal
Sexual Functioning	Sexual sensitivity Potential for orgasm Capacity for intercourse, if desired
Psychological adjustment ("quality of life")	Happiness Absence of distress Satisfaction with specific aspects of life e.g., psychosexual adjustment

complained that surgery does not prevent problems and may actually exacerbate them, because of adverse cosmetic and functional outcomes from surgery. These critics further contend that problems arise from the undue focus on the genitalia and not their appearance per se.

The surgical outcomes most often studied have been genital appearance and adequacy of genitalia for peno-vaginal intercourse. But the assumptions behind surgery and the concerns of patients make it clear that other outcomes need to be considered, particularly those related to the quality of sexual experience, including sensitivity and satisfaction, and general quality of life (Table 3).

Physical Outcomes of Surgery

There are no systematic outcome data regarding genital appearance and sexual function, especially for current surgical procedures. There are reports of suboptimal cosmetic outcome and self-reported sexual function, but they are based on limited assessments of selected patients with surgery of varying quality.[26,27,31] Therefore, it is difficult to know how surgery affects sexual function, and the factors that account for variations across individuals. Measures of clitoral responsivity suggest normal nerve conduction after surgery,[32] but it is unclear whether this translates into normal sensitivity. It is also important to remember that intercourse is only one part of sexual activity, and surgery to facilitate intercourse might compromise orgasmic response.

There is optimism that current techniques used by skilled surgeons produce better cosmetic and functional outcomes now than in the past,[33] but confirming evidence is essential. Outcome studies require detailed assessments and comparisons with subjects without intersex conditions, given the complexity of sexual response, the variations in arousal and orgasm among typical individuals without genital surgery,[34] and the limitations of self-report in assessing sexual response.[35]

Psychological Impact of Genital Appearance

Both physicians and intersex advocates are concerned about psychological problems associated with intersexuality. Physicians suggest that children who look different will have difficulty forming a coherent self-concept, including gender identity, and receive negative reactions from others, with adverse effects on adjustment and life satisfaction. Some intersex advocates argue that problems result from stigma and shame induced by messages from physicians and parents that atypical genitalia are unacceptable.

Neither set of concerns have been empirically validated – or refuted. There are no data showing the relative importance or unimportance of normal-appearing genitalia for psychological outcome. The existence of gender dysphoria in individuals with and without intersex conditions indicates that normal-appearing genitalia are not sufficient for gender identity consistent with rearing sex, but there is no systematic study of the role (if any) that genital appearance plays in the development of gender identity. It is widely believed that boys with a small penis are teased, causing poor peer relationships and adjustment problems. Although this has not been systematically studied, males with micropenis appear to do well.[24,25] Relevant data from boys with hypospadias who had received genital surgery show psychological adjustment similar to that of control boys, with little relation between adjustment and genital appearance, but depression is associated with more surgery and hospitalizations.[36]

Evidence from individuals with other physical conditions reinforces the complex contributors to outcome. Problems in individuals with intersex conditions might not arise from specific aspects of the condition or treatment itself, but from the stresses they impose on the patient and the family.[37] Children's stress may arise from their own experiences, such as surgery, repeated physical exams and hospitalizations, responses to their unusual genital appearance, or from changes in parent-child interactions brought about by parents' stress. Parent stress may be independent of the child's physical illness or may result from it, for example, from concerns about the child's genital appearance, responsibilities of caring for a sick child, or financial burdens brought about by the child's illness. Additional risk may arise from children's problems with peer relationships,[38] but even here the cause is not simple. Peer problems are affected by more than physical appearance, such as frequent school absences and sex-atypical behavior.[37,39] Furthermore, the association between peer relationships and adjustment is bidirectional: poor peer relations place a child at psychological risk, but poorly adjusted children have difficulty making friends to start.

Psychological Outcome in Intersexuality

Thus, there are many paths by which mental health might be affected in individuals with intersex conditions, but there is no evidence regarding any of them. Further, there is surprisingly little evidence about the ultimate mental health outcomes hypothesized to be affected by these paths, primarily because such studies are difficult. Scientific studies may undersample individuals with problems, but reports from intersex activists may overrepresent those with problems.[40]

The most systematic evidence regarding mental health in intersex individuals comes from females with CAH. Several studies show that their mental health is not different than that of controls, although they may have specific problems with body image and psychosexual function.[41-46] There are not enough data to know whether outcome is related to genital appearance or surgery.

These results on good adjustment might be surprising in light of assumptions described above. However, they are consistent with evidence that chronic illness, trauma, and other adverse life events have only transient effects on adjustment in the majority of people. Among individuals with a variety of physical disabilities (including quadriplegia), there is often an immediate period of depression, but after a short period (weeks to months), most report positive well-being.[47,48]

This mismatch between expectation and evidence is an example of the tendency to attribute outcome to the cause that is most salient, in this case, the appearance of the genitalia or the intersex condition itself. But, outcome is influenced by many factors, including temperament and life circumstances. People are not accurate at predicting factors that influence life satisfaction in others because they only focus on a small set of contributors.[49] This means that attributions about problems among intersex individuals must be validated empirically.

Recommendations Regarding Surgery

The lack of systematic outcome data makes decisions about genital surgery very difficult. There are insufficient data regarding the functional consequences of genital surgery, but there are also insufficient data regarding the effects on a child of living with atypical genitalia. It is likely that the effects of both genital surgery and genital appearance are not the same for all individuals. Perceptions of and responses to the situation may be more important than its objective nature, and psychological support may help families develop coping strategies to foster mental health. It is important to remember that decisions should be made in the best interests of the child and not the parents.

CONCLUSIONS

The discussions surrounding the treatment of children with intersex conditions have crystallized the assumptions and evidence underlying treatment. Changes to treatment must be informed by evidence or, consequently, dilemmas will arise again. Despite gaps in the evidence regarding outcome, there is some information available to guide treatment.

First, sex assignment cannot be based on the assumption that gender identity is determined by either sex of rearing or degree of fetal androgen exposure. Most individuals with 46,XX CAH do well when reared as girls, but there are no systematic studies of those reared as boys. Most individuals with 46,XY micropenis appear to do well when reared as boys, but this approach should be viewed cautiously until there is more evidence about psychological and sexual outcome with male vs. female rearing. There is insufficient evidence regarding other causes of intersexuality and cloacal exstrophy, but all children should be assigned as girls or boys, with the recognition that some may change gender later in life.

Second, decisions about surgery would benefit from systematic evidence regarding functional outcome of current procedures and consequences of atypical genitalia. Sexual function involves more than cosmetic appearance and the ability to have intercourse. Given the dearth of evidence, assumptions and biases should be clearly articulated to families.

Third, there is a pressing need for additional systematic evidence that addresses the complex determinants of psychological outcome. It is not sufficient to examine outcome only in relation to characteristics of the intersex condition and its treatment. There must be recognition and consideration of the child's temperament, family situation, culture in which the child lives, and benefits of psychoeducational interventions to reduce stress and facilitate coping.

Outcome itself must be defined from the perspective of the patient, and include quality of life. The components of outcome are not interchangeable (Table 3).

Fourth, translation of findings to treatment requires that studies meet important methodological criteria regarding sampling, assessment, and inferences consistent with the limitations of the methodology (Table 4). It is important to avoid being swayed by studies that support preconceptions or provide simple solutions.

Recent debates have improved treatment of children with intersex conditions by forcing an articulation of assumptions and examination of evidence. Resolution of current controversies requires a commitment to

Table 4

Considerations in Evaluating Outcome Studies of Children with Intersex Conditions

Sampling
What was the population sampled?
What proportion of potential participants were studied?
How do the participants compare to the nonparticipants?
How would results change if nonparticipants have different outcome?
What was the comparison group?
Were the samples of intersex and comparison individuals large enough to see effects of clinical significance, including group differences and predictors of outcome?

Outcome Assessment
Were different aspects of outcome carefully differentiated?
For example, was gender identity measured independently of gender role?
Was each outcome assessed in detail with reliable and valid measures?
Were patients compared to controls to be sure that outcome is specific to an intersex condition?
Were hypothesized predictors of outcome assessed in detail with reliable and valid measures?

Inferences
Were appropriate statistical comparisons made so that inferences can be made to the population?
To what populations can results be generalized?
Can outcome be empirically attributed to intersex condition itself?
Can outcome be empirically attributed to specific factors related or unrelated to intersex condition?
Are inferences appropriately qualified in light of (inevitable) methodological limitations?

evidence-based care and a recognition that outcome in intersexuality cannot be simply predicted from medical factors alone.

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Commentary: Intersex Issues - A Series of Continuing Conundrums

Dr. Blizzard has abstracted and commented upon two extraordinarily important manuscripts by Migeon and colleagues. These investigators have provided the first analysis of the long-term outcome of 75 adults with male pseudohermaphroditism or micropenis (46XY or 45X/46XY) managed as children at Johns Hopkins Hospital. These children had been assigned to either the male or female gender. All of 18 patients with feminine external genitalia (androgen insensitivity syndrome or complete gonadal dysgenesis) were raised as females; 5/18 subjects with micropenis (stretched length <1.9 cm) without hypospadias were reared as females. In 39 subjects with ambiguous genitalia, 18 of whom were raised as female and in whom in depth information concerning their "sexuality" was sought, the assigned sex was at least "satisfactory" in the majority. Indeed, those reared as male had greater incidence of atypical external genitalia and greater dissatisfaction with perceived "body image". In general, however, the outlook for normal adult heterosexual adjustment reared as either male or female was quite good in this group.

Until more complete data are available, these observations can serve as the basis upon which to counsel the parents of a neonate with male pseudohermaphroditism in regard to their choice in the gender assignment of their offspring. Dr. Blizzard correctly states that the "paternalistic" approach to medical practice is no longer tenable.

In my opinion, in the context of this psychosocial emergency, it remains extremely important that the experienced physician assist, perhaps even guide, the parents through the decision making process. In the absence of androgen insensitivity, complete gonadal dysgenesis, deficiency of P450$_{side\ chain\ cleavage}$ or 17-hydroxylase/17-20 lyase, and related disorders, it seems most appropriate to rear the incompletely virilized male in the masculine gender if there is at all sufficient penile corpus to do so or to permit its surgical amplification.

Dr. Blizzard critically analyzes the current thinking concerning the problem of when to perform reconstructive genital surgery in the patient with male pseudohermaphroditism assigned to the female gender.

In my opinion, he correctly rejects the extremist position that no reconstruction be undertaken until the patient herself can consent. Clearly, this approach will lead to great duress in the lives of the patient and her parents. (One can barely imagine the stress that a parent would be under in raising a child whose gender may change or that of the child who will surely learn at a surprisingly early age that her genitalia differ from those of other girls.) While each child must be considered individually, cliteroplasty during infancy and vaginoplasty at adolescence seem reasonable in my opinion once feminine gender has been assigned until the long-term efficacy of earlier vaginal reconstructive techniques have been evaluated.

Dr. Blizzard discusses the issue of intra-cultural differences in attitude toward the problem of intersex and the challenging question of whether all children with 46XX female pseudohermaphroditism should be reared as females.

His thoughtful and insightful comments are seconded by this writer, although my inclination is to rear all females with virilizing congenital adrenal hyperplasia as girls. Individualization of care and informed parental choice are the keystones upon which management of the neonate with atypical external genitalia must be based.

Readers who wish to be brought up-to-date concerning some of the conundrums of intersex issues and what the current concepts are concerning intersex issues will benefit from Dr. Blizzard's commentary.

Blizzard RM. *Pediatrics* 2002;110(3):616-621.

Allen W. Root, MD

Dr. Blizzard's Comment: *Comments about one's commentary are not necessarily legitimate. However, I comment relating the above abstract and editorial comment by Dr. Root to the lead article in this issue by Dr. Sheri Berenbaum. Her studies and writings are always logical, intelligent, and scientifically based. In her article, Dr. Berenbaum demonstrates the applicability of my adjectives used to describe her approaches to solving the conundrums of intersex. I highly recommend each reader contemplate her description of the complexities in this field. Hopefully others will approach the conundrums of intersex in the same contemplative way as does she.*

Robert M. Blizzard, MD

Letter to the Editors:

In the December 2002 edition of *Growth, Genetics & Hormones* (Vol. 18. No. 4), two articles (Imaizumi K, et al. *Am J Med Gent* 2002;107:58-60; Kurotaki N, et al. *Nat Gen* 2002;30:365-366) were abstracted under the title *A Gene as a Major Cause of Sotos Syndrome Has Been Identified.* The authors are reported to state that the identification of a deletion or mutation of this mutated gene on chromosome 5 will sometimes help in the diagnosis of Sotos syndrome, etc. Both Dr. Judy Hall and Dr. William Horton gave cogent editorial comments.

However more recent evidence indicates that additional knowledge gained by Kurotaki and others should be considered by clinicians and investigators attempting to use identification of a deletion or mutation of this mutated gene (NSD1) to help in the diagnosis of Sotos syndrome. Specifically, at the ASHG meeting in October 2002, Kurotaki et al from Japan reported finding point mutations and deletions of the NSD1 gene in a large series of patients and Clech et al from Paris reported their findings in 39 patients. Only 14 were felt to have typical Sotos syndrome; four had a NSD1 deletion of paternal origin. It had previously been suggested that based on similarity of the phenotypes, Sotos and Weaver syndromes might be allelic disorders. Rahman et al from the UK reported that >40% of patients with typical Sotos syndrome had intragenic mutations in NSD1 and 3 of 7 patients with Weaver syndrome had intragenic NSD1 mutations. In each of these series, patients with a combination of overgrowth and mental retardation, but without typical features of either Sotos or Weaver syndrome, were not found to have deletions or intragenic mutations of NSD1.

These reports collectively demonstrate that the majority of patients with typical Sotos and Weaver syndrome have intragenic mutations or deletions of NSD1, and thus, represent allelic disorders. However, the combination of overgrowth and mental retardation represents a heterogeneous phenotype in which only a portion is accounted for by abnormalities of NSD1.

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Editorial Comment:

Sotos syndrome and Weaver syndrome are both overgrowth syndromes beginning usually prenatally. Such overgrowth continues during childhood. These two syndromes are similar in many respects; in respect to overgrowth, mental retardation, large hands and feet, advanced bone age, and tall stature but, usually, adult height within the normal advanced percentiles. However, they do differ in certain subtle respects. The patient with Sotos syndrome (cerebral gigantism) has a head that is dolichocephalic. The occiput tends to be flat in the patients with Weaver syndrome. The face tends to be smaller. There are hypoplastic facial bones and macrognathia in Weaver syndrome, but pointed chin and normal mandibular development prompts one to think more of Sotos syndrome. The joints are limited in motion often in Weaver syndrome with limited elbow, ankle, wrist, hip, and knee extension. The long bones are widened or splayed in Weaver syndrome and camptodactyly is frequent. Further details concerning these two syndromes can be pulled from the pediatric database, although the update listed is 1994 (http://www.icondata.com/health/pedbase/files/sotosynd.htm - or - weaversy.htm. Comparable data can also be found on the web at http://www.nlm.nih.gov. At this web site you will have a choice to enter "Weaver".

Robert M. Blizzard, MD

Abstracts from the Literature

Circulating Levels of IGF-1 Directly Regulate Bone Growth and Density

Previous studies by LeRoith and co-workers and Ueki et al have demonstrated that selective loss of liver-derived insulin-like growth factor-1 (IGF-1) or of acid labile subunit (ALS) does not substantially impair murine growth and development despite marked decline in circulating levels of IGF-1.[1,2] This has led to the suggestion that only the IGF-1 produced locally by bone is necessary for linear growth.[3]

In order to explore this question further, LeRoith and his colleagues developed double "knock-out" animals which were deficient in both liver IGF-1 and ALS (LID-ALSKO), and compared these with animals deficient only in liver IGF-1 (LIDKO) or ALSKO. As anticipated, serum concentrations of IGF-1 were decreased markedly, -65% in ALSKO, -75% in LIDKO, and -90% in LID-ALSKO relative to control animals with normal hepatic IGF-1 and ALS production. However, the rate of IGF binding protein-3 (IGFBP-3) degradation was also increased in these animals; thus free IGF-1 values were increased modestly in LIDKO (+150%), minimally in ALSKO (+108%), and markedly in LID-ALSKO animals (+350%). Growth hormone and insulin concentrations were greatly increased in LID-ALSKO mice. The clearance of IGF-1 was markedly accelerated in ALSKO (32 minutes) and LID-ALSKO (18 minutes) as compared with control (69 minutes) and LIDKO (73 minutes) mice, reflective of lack of binding of IGF-1 to IGFBP-3/ALS.

Intrauterine growth of all animals was apparently normal. By 3 weeks and 4 weeks of post natal age (Figures), the length and weight of the LID-ALSKO mice were less than those of the intact animals. Linear growth of the LIDKO and ALSKO animals did not differ from controls. However, the rate of weight gain of ALSKO mice was impaired to the same extent as that of the LID-ALSKO group. Tibial length, and heights of germinal, proliferating, and hypertrophic zones of the proximal tibial growth plate, were significantly diminished in the LID-ALSKO mice but not in the two single "knock-out" groups. On the other hand, femoral length, total and cortical bone density, periosteal circumference, and cortical and trabecular bone volume were diminished in all "knock-out" groups, but to a substantially greater degree in the LID-ALSKO animals. Administration of exogenous IGF-I increased linear growth, femoral length, and size of the proximal tibial growth plate, as well as IGFBP-3 concentrations, in all groups. IGF-1 mRNA levels in bone were similar in all groups.

The investigators concluded that *circulating* IGF-1 was important for linear and appositional bone growth and bone mineralization and that its effects were mediated through actions on periosteal osteoblasts as well as upon chondrocytes within the epiphyseal growth plates.

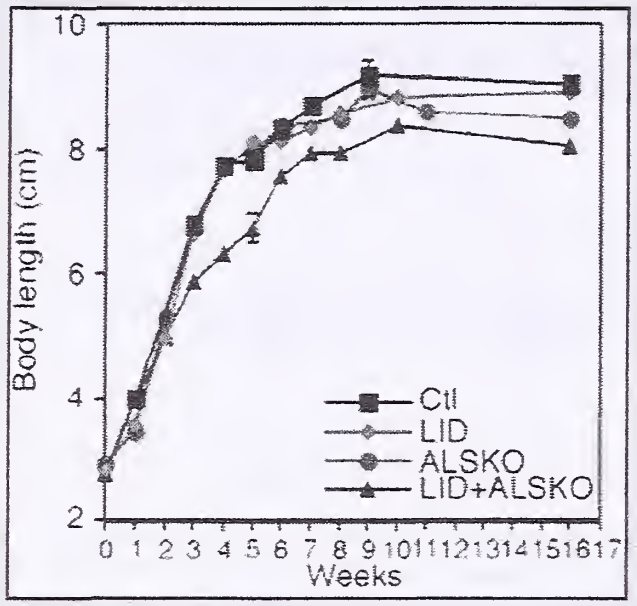
Yakar S, et al. *J Clin Invest* 2002;110:771-781.

***First Editor's Comment**: This important paper establishes the necessity of circulating IGF-I for normal growth and bone mineralization. It demonstrates that osseous synthesis of IGF-I alone is insufficient for normal linear growth of bone and mineral deposition. Thus, reexamination of the "somatomedin hypothesis" suggests that both liver derived and locally synthesized IGF-I are necessary for normal bone metabolism. Interestingly, "knock-out" of any of the IGFBPs has little effect upon the phenotype of the mutant mouse, but their over expression results in inhibition of growth.[4] One wonders what the phenotype of the mouse that lacks IGF-I, IGFBP-3, and ALS might be ... possibly lethal?*

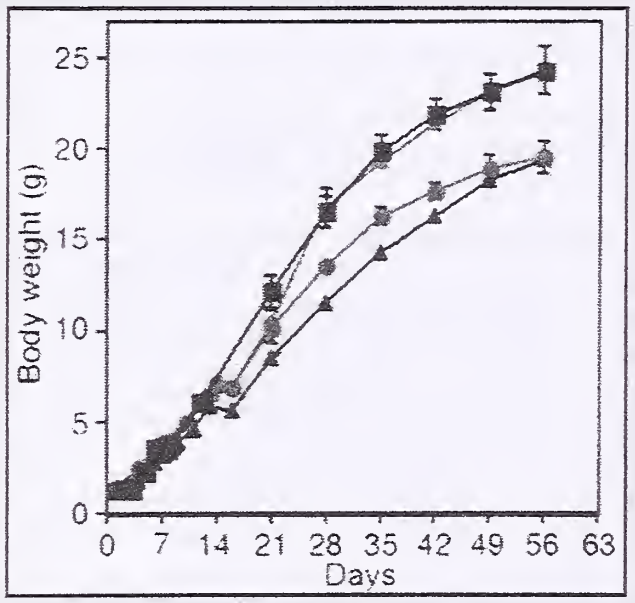
Allen W. Root, MD

Figures

Postnatal growth in LID+ALSKO mice



Body length was measured from nose to anus at weekly intervals (n = 20-30 mice per group).



Body weight was measured at weekly intervals from birth to the age of 8 weeks (n = 30-60 mice per group).

References

1. LeRoith D, et al. *Endocrine Rev* 2001;22:53-74.
2. Ueki I, et al. *Proc Natl Acad Sci USA* 2000;97:6868-6873.
3. Kaplan SA. *Growth Genetics & Hormones* 2002;18:38-39.
4. Silha JV, Murphy LJ. *Endocrinology* 2002;143:3711-3714.

Second Editor's Comment: *In Growth, Genetics & Hormones (Vol. 18, No. 3), an important lead article entitled Somatomedin Hypothesis: Time for Reexamination was written by Dr. Solomon Kaplan. He has been asked to write an editorial comment.*

Dr. Kaplan's Comment: *The paper by Yakar et al extends and amplifies the findings in a previous publication by the authors[1] on the role of circulating IGF-1 in promoting longitudinal growth in mice. They had already shown that despite inactivation of the IGF-1 gene in the liver, resulting in reduced concentrations of circulating IGF-1 by as much as 75%, the growth of the animals was not impaired. Their findings were consistent with the growing body of evidence against the validity of the somatomedin hypothesis, which holds that the effects of growth hormone on longitudinal growth are mediated through hepatic production of IGF-1.[2]*

IGF-1 circulates in the serum largely as a 150-kDa complex comprised of the IGF-1 molecule, IGF binding proteins (mostly IGFBP-3), and the acid labile subunit (ALS). Others had previously shown that ALS knockout (ALSKO) mice experienced only mild growth retardation despite profound disruption of the circulating IGF system.

Yakar's current paper reported the effects of double gene disruption of the IGF system: inactivation of the hepatic gene for IGF-1 (LID) combined with ALSKO, on bone growth and density. In the mice carrying the double gene deletion, there was a reduction of circulating IGF-1 concentrations by as much as 85 to 90%; the animals also experienced significant growth impairment. There was a diminution in the amount of circulating IGFBP-3 protein and also in the free IGF-1 fraction. Loss of ALS led to more rapid disappearance of 125-I labeled IGF from the serum because absence of the ALS protein

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leads to proteolytic cleavage of IGFBP-3 and loss of its protective binding of IGF-1. The authors conclude that a minimum concentration of IGF-1 in the serum, higher than what they observed in the double gene-deletion mice, is necessary for normal bone and somatic growth.

Following administration of IGF-1 by injection, the animals with the double gene deletion experienced increased serum IGF and IGFBP-3 concentrations accompanied by restoration of normal bone growth and modeling, as well as increased somatic growth. These findings are consistent with their observation that the restoration of normal growth can be accounted for by increased serum IGF-1 concentrations above the minimal levels necessary for normal growth to occur.

This paper provides confirmatory evidence that hepatic derived IGF-1 and acid labile subunit are not necessary for normal growth provided minimal serum levels are maintained from non-hepatic sources including autocrine/paracrine production by target tissues.

Solomon A. Kaplan, MD

References

1. Yakar S, et al. *Proc Natl Acad Sci USA* 1999;96:7324-9.
2. Daughaday WH, et al. *Nature* 1972;235:107.

The *BRCA2* Gene's Role in Fanconi Anemia and Various Cancers

Fanconi anemia (FA) is an autosomal recessive disorder in which affected subjects have great susceptibility to neoplasia early in life, including acute myeloid leukemia and squamous cell carcinoma. Bone marrow failure is also frequent, as well as mutations in at least 8 groups of FA patients (A, B, C, D_1, D_2, E, F and G) and germline mutations in six of these have been identified in 6 genes (A, C, D_2, E, F and G). The FA cells manifest many broken and misshapen chromosomes reflecting that FA proteins participate in the repair of DNA damage, either stimulating or inhibiting normal repairs. Five of the 6 genes previously described combine in a multi-subunit nuclear complex which activates by ubiquitination of the protein product of a sixth gene (FANCD2) which is involved in the process of DNA repair. Howlett et al[1] identified a 7th gene by demonstrating that homozygous "loss of function" mutations occurring in the BRCA2 gene (causing breast cancer as does the BRCA1 gene) occurs in a subset of patients with FA.

Witt and Ashworth[2] stated in the introduction of their commentary; "Important discoveries are so neat and satisfying that, in retrospect, they seem obvious. Howlett et al disclosed that the inheritance of two defective copies of the BRCA2 breast cancer susceptibility gene can lead to FA. The BRCA2 protein is thought to be important in the repair of DNA damage. Cells lacking BRCA2 inaccurately repair damaged DNA leading to gene mutation and progression of tumors and are particularly sensitive to DNA cross-linking agents. Howlett et al demonstrated that one of the previously unidentified FA genes (FANCD1) is BRCA2." No BRCA1 mutations were found in the patients studied by Howlett et al. However, all the authors of all three papers speculatively agreed that the 6 previously cloned genes are linked in a common pathway with BRCA1 and BRCA2 genes.[1-3]

Venkitaraman[3] in his closing comments stated; "The network which connects BRCA and FA proteins in DNA repair includes at least two other molecules - ATM (mutated in ataxia telangiectasia) and CHEK2 - whose inactivation is also associated with carcinogenesis in several tissues. Although the precise functional connections between the molecules in this network remain obscure, it is clear we are glimpsing an important tumour suppressor pathway whose disruption may underlie many different types of human cancer."

1. Howlett NG, et al. *Science* 2002;297:606-609.
2. Witt E, Ashworth A. *Science* 2002;297:534.
3. Venkitaraman AR. *Lancet* 2002;369:1343-1345.

First Editor's Comment: *Heterozygous inactivating germline mutations in BRCA1 and BRCA2 have been linked to increased susceptibility to breast and ovarian cancer in women.[1] In the tumors that develop in these patients, there is loss of heterozygosity of BRCA1 or BRCA2. Both BRCA1 and BRCA2 are important for repair of DNA damaged by exposure to ionizing radiation and cross-linking, and do so by interrupting the cell cycle while promoting repair of the damaged DNA strands.[1-3] The carboxyl-terminal domain of BRCA2 likely binds to single strands of DNA at the site(s) of a double stranded DNA break and facilitates the binding of other repair factors such as RAD51, an important member of this family. This article is of interest because it demonstrates the difference in phenotypes that result from heterozygous as compared to homozygous germline mutations in BRCA2. How this mutation affects somatic growth and the reproductive endocrine system is unclear. However, Wajnrajch et al[4] found aberrations of endocrine function in 44/54 primarily prepubertal patients with FA.[4] Abnormalities included short stature with mean height SDS -2.35 (due to growth hormone insufficiency in 44%), hypothyroidism (36%), hyperinsulinemia (72%), impaired glucose tolerance (25%), and diabetes mellitus (2%). Skeletal maturation*

was approximately one year delayed behind chronologic age; predicted adult height in 22 subjects was -1.24 SDS.

References

1. Wilson JH, Elledge SJ. *Science* 2002;297:1822-1823.
2. Yang H, et al. *Science* 2002;297:1837-1848.
3. Witt E, Ashworth A. *Science* 2002;297:534.
4. Wajnrajch MP, et al. *Pediatrics* 2001;107:744-754.

Allen W. Root, MD

Second Editor's Comment: *The phenomena described in the papers given as references are phenomenal. The first 3 references read as a package will permit any reader not informed about such matters to advance into the upper elementary levels, both in respect to understanding the physiology and pathophysiology of Fanconi Anemia, breast cancer, and to the interactions of genes and gene products.*

Robert M. Blizzard, MD

Serum Zinc in Infants and Preschool Children in the Jeddah Area: Effect of Diet and Diarrhea in Relation to Growth

Dr. Bahijri has written a thoughtful analysis of the etiology and effect of zinc deficiency on wasting and stunting of 728 children in 5 age groups (4-6, 6-<12, 12-<24, 24-<36, and 36-72 months). Using the concept of weight for height, the subjects were classified according to their grade of wasting, and using the concept of height for age, the subjects were classified according to their grade of stunting. The dietary, auxological, and chemical evaluations were carefully done in accord with the most modern standards and techniques. The study was undertaken to determine the prevalence of zinc deficiency in the Jeddah (Saudi Arabia) area among preschool age children, to see whether such a deficiency is a cause of retarded growth, to determine whether a relationship exists between height for age and serum zinc concentrations, and if possible to determine the causes of zinc deficiency.

The authors presented serum zinc levels in the various age groups for subjects: (1) without stunting and wasting, (2) with various grades of wasting, (3) with various grades of stunting, and (4) with both stunting and wasting. Many subjects in each group had zinc levels <10.4 umol/L which is frequently cited in the literature as the cut off for normalcy. However, the lowest mean serum zinc levels were found in the patients in the group with stunting and wasting. Whereas those who had neither stunting nor wasting had the highest levels. The older stunted children (group 3) had lower zinc levels than those found in the younger children. All patients with wasting (group 2) had hypozincemia.

The authors concluded that diarrhea rather than low dietary intake mostly accounts for the low zinc levels in infants (4-12 months). As the subjects passed the 24 month mark, diet deficiency became the presumed major cause of hypozincemia and this cause became more dominant as the etiology in the oldest age group (36-72 months).

The importance of zinc in biology is well reviewed, including that zinc is known to influence cell division, growth and development, as well as sexual maturation. It is needed also as a membrane stabilizer, and is essential for the integrity of the immune system. More than 100 enzymes require zinc as a cofactor, and zinc seems to be involved in the proper storage and release of insulin, growth and repair of tissues, wound healing, ability to taste food, production of prostaglandins, mineralization of bone, blood clotting, function of vitamin A, and functions of the thyroid hormones.

Not commonly known, an important predisposing factor for zinc deficiency is the extensive use of cereal protein which limits the availability of zinc due to high phosphate and phytate content. The recommended dietary allowance of the Food and Nutrition Board and the National Academy of Sciences in the United States is 15 mg/day for adult males and 12 mg/day for adult females, with higher recommended levels during pregnancy and lactation. Requirements for infants and children are relatively high in relation to body size because of increased requirements for physical growth.

The best sources for zinc in the diet are meat and fish; the bioavailability of zinc from animal products is considered to be greater than that from plants. Diarrhea is associated with zinc deficiency and low serum zinc concentration. Suggestions have been made that growth retardation commonly seen in children in developing countries is related to zinc nutritional deficiency.

Unfortunately, it was not feasible to interpret the direct effect of zinc deficiency on wasting or stunting although a significant majority of subjects with wasting and/or stunting had severe deficiency. The author summarized: "The result of this work shows a high incidence of low serum zinc levels among Jeddah-area infants and young preschool children, which is associated with diarrhea and wasting in the first two years of life, and generally low dietary intake, wasting and/or stunting in older children. Zinc supplementation is recommended for certain categories of subjects to improve appetite and hence dietary intake, immunocompetence, and anthropometric measurements."

Bahijri SM. *Annals of Saudi Medicine* 2002;21:324-329.

First Editor's Comment: *A complete reprint of this article will be sent to those who request it by e-mail to rblizzard@compuserve.com.*

Unfortunately in nearly all studies of this type it is difficult to separate cause and effect. For example, does malnutrition or illness produce wasting and/or stunting accompanied by zinc deficiency or is the zinc deficiency etiologic in malnutrition and/or illness and/or stunting and/or wasting? In spite of this excellent study, the answer to this question remains an enigma. Moreover, zinc supplementation seems indicated to a much greater extent than currently in use.

Robert M. Blizzard, MD

Second Editor's Comment: *Recently Brown et al[1] published a meta-analysis of randomized controlled trials of the effects of supplemental zinc on the growth and serum concentrations of prepubertal children. A total of 33 studies were compiled demonstrating that zinc supplementation produced a significant positive height response and an increase in serum zinc levels. Growth responses were greater in those children with low weight for age and low height for age. This paper was reviewed in Growth, Genetics & Hormones in 2002 (Vol. 18, No. 4) and the importance of recognizing the value of zinc nutriture in "at risk" populations was emphasized. However the note of caution noted below by Dr. Tarim should be kept in mind.*

Fima Lifshitz, MD

Reference

1. Brown KH, et al. *Am J Clin Nutr* 2002;75:1062-1071.

Letter to the Editor:

I would like to add a precaution before suggesting zinc supplementation to anyone with nutritional growth retardation who lives in places where zinc deficiency may be prevalent. Iron deficiency which may co-exist with zinc deficiency may be aggravated during zinc therapy because these two minerals may block the intestinal absorption of each other.[1] Consequently, iron deficiency may also worsen growth retardation. Therefore, I suggest excluding iron deficiency, which is easier to diagnose than zinc deficiency, before initiating zinc supplementation.

Omer Tarim, MD
Director of Pediatric Endocrinology
Uludag University Faculty of Medicine
Bursa, Turkey

Reference

1. Lifshitz F, et al. Nutritional Growth Retardation. In: Lifshitz F, ed. *Pediatric Endocrinology 3rd Edition*. New York: Marcel Dekker, 1996:103-120.

Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus

Because multiple laboratory tests are used in the diagnosis and management of this disease, the quality of the scientific evidence supporting the use of these assays varies. Therefore, an expert committee drafted evidence-based recommendations for the use of laboratory analysis in patients with DM. An external panel of experts (DB Sacks, DE Bruns, DE Goldstein, NK Maclaren, JM McDonald and M Parrott) reviewed a draft of the guidelines, which were modified in response to the reviewers' suggestions, and other steps were taken to gain a consensus of expert opinions. The guidelines, as published in Clinical Chemistry, consist of an Executive Summary of one page providing specific recommendations based on data published or expert consensus. Several analyses are of minimal clinical value at the present time and measurement of them is not recommended. The entire article is 42 pages. Those clinicians treating diabetics should at least scan the article and closely scrutinize the Executive Summary.

Highlignts of the Executive Summary are now presented:

Glucose should be measured in an accredited laboratory to establish the diagnosis of DM and to screen high-risk individuals. Blood should be drawn after an overnight fast. Glucose should be measured in plasma. If plasma cannot be separated from cells within 60 minutes, a tube with glycolytic inhibitor should be used. On the basis of biological variation, glucose analysis should have analytical imprecision less than 3.3%, bias less than 2.5%, and total error less than 7.9%.

The OGTT is not recommended for the routine diagnosis of type 1 or 2 DM. The key limitation of the OGTT is its poor reproducibility. It is recommended for establishing the diagnosis of gestational DM.

Because of the imprecision and variability among glucose meters, they should not be used to diagnose DM and have limited value in screening. Noninvasive glucose analyses cannot be recommended at present as replacements for plasma glucose or measurements by an accredited laboratory. Glycated hemoglobin (GH_B) should be measured at least biannually in all patients with DM. US laboratories should use GH_B assays certified by the National GH Standardization Program

(NGSP) as traceable to the DCCT reference. GH_B levels should be maintained at <7% and the treatment regimen should be reevaluated if GH_B is >8% as measured by NGSP - certified methods.

Routine measurement of genetic markers is not recommended for the diagnosis or management of patients with DM. Likewise, autoimmune markers lack specificity and are not recommended for routine diagnosis or screening of DM.

An annual search for micro albuminuria should be performed on patients without clinical proteinuria. To be useful, semiquantitative or quantitative screening tests must be shown to be positive in >95% of patients with micro albuminuria. Positive results must be confirmed by quantitative testing in an accredited laboratory.

All adults with DM should receive annual lipid profiles.

Sacks DB, et al. *Clinical Chemistry* 2002;48:3,436-472.

***Editor's Comment**: This is only the very essential infrastructure of the Executive Summary. The article is endowed with significant substance.*

Robert M. Blizzard, MD

Mutations of the *Great* Gene Cause Cryptorchidism

The investigators previously identified a mutant strain of mice (*crsp*) with high intraabdominal bilateral cryptorchidism due to a 550 kb deletion of the proximal arm of mouse chromosome 5. Within the deleted region, the investigators identified a G-protein coupled receptor gene (GPCR) termed "G-protein coupled receptor affecting testis descent" or *Great*. *Great* was expressed in testis, brain, and skeletal muscle. In the current paper, the authors developed a mouse "knock-out" model of this gene. The phenotypes of the wild type mice and those who were heterozygous (*Great*$^{+/-}$) were normal. However, animals who were homozygous for the mutation (*Great*$^{-/-}$) were similar in phenotype to *crsp* mice. In (*Great*$^{-/-}$) mice, there was failure of development of the gubernaculum (the ligament whose shortening is partially responsible for the inguinal-scrotal phase of testicular descent). The investigators then cloned human *GREAT* (chromosome 13q12-13), an 18 exon gene encoding a GPCR, and analyzed its structure in 61 men with bilateral (N=31) or unilateral cryptorchidism. In one subject with bilateral cryptorchidism, a heterozygous loss-of-function mutation was identified (exon 8, A C, Tyr222Pro was identified). The authors concluded that mutations in *GREAT* are responsible for cryptorchidism in some human males but the frequency of a *GREAT* as a cause of cryptorchidism mutation remains to be determined.

Gorlov IP, et al. *Hum Molec Genet* 2002;11:2309-2318.

***First Editor's Comment**: GREAT had been cloned by other workers and termed LGR8 - Leucine-rich repeat-containing GPCR. Relaxin had been identified as a ligand for GREAT. However, testicular descent is normal in the Relaxin "knock-out" male mouse. Insl3 - insulin-like factor 3 - is a member of the relaxin family and is synthesized in the testes; its loss results in bilateral cryptorchidism due to maldevelopment of the gubernaculum. Thus, Insl3 may be the natural ligand for GREAT. While homozygous loss of Great is needed for cryptorchidism in mice, apparently its heterozygous loss appears to be sufficient in humans to cause this malformation; the mechanism(s) of this species difference is/are not defined at present.*

There are two phases of testicular descent - transabdominal and inguinal-scrotal. The first phase is conditioned by failure of development of a cranial suspensory ligament mediated by testosterone. The second phase is stimulated by development of the gubernaculum, demonstrated to be related to the interaction of Insl3 and GREAT. Mullerian duct inhibitory factor and its receptor also play a role in this phase of testicular descent. The manuscript also suggests that it would be inappropriate to tell another gentleman that he is "not so GREAT!"

Allen W. Root, MD

References

1. Overbeek PA, et al. *Genesis* 2001;30:26-35.
2. Nef S, Parada LF. *Nat Genet* 1999;22:295-299.
3. Teixera J, et al. *Endocrine Rev* 2001;22:657-674.

***Second Editor's Comment:** This article is the best I have read concerning the development and descent of the testes. Work in mice and in humans is blended in describing the embryological development of both testes and ovaries. The 11 authors come from diverse and multiple fields - urology, genetics, pharmacology, embryology, molecular biology, etc., which largely accounts for the excellence of the article. Those interested in gonadal development, normal and/or abnormal, will be gratified in reading the article in its entirety.*

Robert M. Blizzard, MD

Kyphosis in Turner Syndrome

Elder and colleagues performed lateral thoracic spine and standing anterior-posterior scoliosis radiographs in 25 of 30 girls between the ages of 5 and 18 years with Turner Syndrome. Excessive kyphosis was defined as an A-P curvature greater than 40%, vertebral wedging as an A-P deformity greater than 5% at any vertebral body, and scoliosis as a lateral curve greater than 10%. Karyotype, age, height, weight, and body mass index percentile, and use and duration of growth hormone, oxandrolone (anavar), and/or estrogen were recorded and entered into a linear regression analysis to determine significant predictors of kyphosis or kyphosis and wedging. Of the 25 subjects studied, 15 (60%) had abnormal radiographic findings. Ten (40%) had excessive kyphosis, 10 (40%) had vertebral wedging, and 5 (20%) had scoliosis. All girls older than 14 years of age (N=8) had excessive kyphosis and wedging.

The subjects were 12.0 ±3.6 years old. Sixty percent had a 45X karyotype, 80% had received GH therapy, and 36% had received estrogen therapy. Logistic regression analysis revealed that chronologic age alone was predictive of excessive kyphosis/wedging, (P=0.053). Stepwise linear regression analysis also showed that chronologic age was predictive of the degree of kyphosis (P=0.032). None of the other variables were predictive. The authors remarked upon the high prevalence of vertebral wedging and excessive kyphosis in their study population. They noted that this is markedly increased compared with the reported prevalence of 3% in the general population. The cause of the scoliosis is apparently multi-factorial, but may include mechanical factors, osteoporosis, adolescent growth spurt, and intrinsic bone defect. Girls with Turner syndrome are known to have a significant number of bony abnormalities, including hypoplasia of cervical vertebrae, and hemivertebrae, although these were not found in the study population. The authors also note that their inability to determine the contribution of age and hormonal therapies to the development of kyphosis may be the result of the small number of subjects studied.

PediaLink.org (Vol. 109) 6/2002. PPE 93.

Editor's Comment: *With such a huge number of Turner subjects (40%) with reported excessive kyphosis, it is surprising that there are not more reports of its prevalence. Indeed this study suggests all girls with Turner syndrome should have routine radiographic screening and should be evaluated by an orthopedist. It is also surprising that more information is not available regarding the probable pathogenesis of these deformities. Since the vast majority of subjects in the study had received or were receiving GH, its contribution to the development of the kyphosis is impossible to determine. However, information from subjects in larger multi-centered databases of individuals who have and have not been treated with GH, would be important to access in order to determine its possible role in the genesis of this deformity. Some information regarding the prevalence of kyphosis in children treated with GH who either had or did not have GH deficiency also could be an important comparison group. Unfortunately this study raises many more questions than it answers, but will probably stimulate other centers to evaluate girls with Turner syndrome. Perhaps a multi-centered survey could help provide a better understanding of this problem. The Growth, Genetics and Hormones Editorial Board welcomes a letter to the editor from readers who have knowledge of data pertinent to the questions raised.*

William L. Clarke, MD

Cancer Risk in Beckwith-Wiedemann Syndrome

Beckwith-Wiedemann Syndrome (BWS) is a well-known syndrome of overgrowth. Macrosomia, neonatal hypoglycemia, midline abdominal defects, macroglossia, ear pits and the predisposition to embryonic cancers in infants and young children, including Wilms tumor, hepatoblastoma and neuroblastoma are the important clinical features of BWS. It is now possible to correlate the phenotypic features with specific genetic disturbances. Most recently, alterations in the imprinting and methylation of several genes in the 11p15 region have been implicated in its etiology. Different patients have different involvement phenotypically and genetically.

De Baun et al have correlated anomalies of DNA methylation of one of the relevant genes, *H19*, in patients with cancer, as compared to those without. Those with cancer are less likely to have abnormalities of the methylation of another gene in the area, *LIT1*. Conversely, abnormalities of methylation of *LIT1* are more likely to be associated with abnormal wall defects and macrosomia. Affected individuals with paternal uniparental disomy of *11p15* are more likely to have associated hemihypertrophy, cancer, and hypoglycemia than those without uniparental disomy.

These findings suggest that all individuals with BWS deserve a precise molecular evaluation in order to be

able to appropriately screen for expected complications. The cluster of genes related to BWS has been studied extensively because of its involvement in the epigenetic phenomenon of imprinting. Abnormal and loss of imprinting of the *IGF2* gene found in this region is present in a number of tumors. *H19* plays a role in the methylation of *IGF2* and so its abnormal methylation or expression may increase the risk of cancer by its relation to *IGF2*.

In the evaluation of BWS, one would expect that cancerous tissue might have different imprinting or methylation than other easier to study tissues. This is particularly frustrating when hemihypertrophy is present. It is interesting to note that any hypertrophy observed in patients with BWS is suggestive of mosaicism. To date, all of the reported patients with paternal UPD of 11p15 are in fact, mosaic. Thus, the two sides of the body probably have different manifestations of the Beckwith-Wiedemann gene cluster.

The hypoglycemia that can be seen in Beckwith-Wiedemann Syndrome also is associated with uniparental paternal disomy. Since hypoglycemia can result in secondary mental retardation, both screening and watching for hypoglycemia in patients with BWS is extremely important during infancy.

DeBaun, et al. *Am J Hum Genet* 2002;70:604-611.

Editor's Comment: *Most of the conditions recognized to be involved in genomic imprinting are associated with abnormalities of growth. Thus, the possibility of genomic imprinting must be considered in any syndrome of abnormal growth. Further evaluation can obviously lead to unique insights about pathogenesis as are being developed in the BWS. This work is allowing recognition of the heterogeneity existing in BWS that may predispose to severe complications.*

Judith G. Hall, OC, MD

GROWTH, Genetics, & Hormones Index for Volume 18 (2002)

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LAWSON WILKINS - PIONEER IN PEDIATRIC ENDOCRINOLOGY AND GROWTH DISORDERS: REVISITED 2003

Robert M. Blizzard, MD
Editor-in-Chief

EDITORIAL INTRODUCTION

In March 1987 in *Growth, Genetics & Hormones*, Vol. 3, No. 1, the lead article with the same title as above was published (the original article is available at the *Archive* section of www.GGHjournal.com). Dr. Wilkins was the founder of pediatric endocrinology. His contributions to pediatrics and pediatric endocrinology were substantial. He was a consummate teacher, practitioner, and investigator, and his personal characteristics were of an exceptional human. He must be known by those who use his name frequently, including members of the Lawson Wilkins Pediatric Endocrine Society and those who utilize his articles in the pediatric literature as references for their own writing. It is for this reason that in this current issue of *Growth, Genetics & Hormones* the article published in *GGH* in 1987 is revisited. In respect to this updating, the two considerations incorporated include an updating of chronological time and the providing of references with highlights concerning Lawson Wilkins as a leader, teacher, pediatrician, and investigator.

Lawson Wilkins, MD

Forty years have passed since 1963, when Dr. Lawson Wilkins died at the age of 69. His demeanor, his accomplishments, and the esteem in which he was held by his peers and his extended family of pediatric endocrine fellows whom he trained are not known to the third and fourth generations of pediatric endocrinologists who are members of the Lawson Wilkins Pediatric Endocrine Society. Since volumes could be written about each aspect of Dr. Wilkins' life, an abbreviated biography is inadequate. Nevertheless, a brief history of Dr. Wilkins' life presents the opportunity to update the image of a man who should be known by pediatric endocrinologists, pediatricians, and geneticists.

Lawson Wilkins was born in 1894 in Baltimore. His father, Dr. George Wilkins, was probably the most highly respected family practitioner in the city. Historical accounts indicate that George Wilkins was intellectually

Highlights In This Issue

Growth, Genetics & Hormones is supported by an unrestricted educational grant from **Genentech, Inc.**

curious, dedicated to his patients, and attentive to detail. His son exhibited the same characteristics. Mrs. Wilkins' death, when Lawson was five years of age, significantly strengthened the already close bond between father and son.

After receiving a baccalaureate degree from Johns Hopkins University in 1914, Lawson Wilkins began medical school there. In 1917, along with many other medical students, he volunteered to go to Europe and served as an orderly in a medical unit during World War I. After the war, he was accepted as an intern in internal medicine at Yale for a year. He then returned to Baltimore to serve a pediatric internship at Johns Hopkins Hospital where the influence of Drs. Blackfan, Park, Kramer, and the other giants of pediatric medicine of the period further whetted his keen intellectual appetite.

It was most likely his desire to follow in his father's footsteps as a practitioner that prompted him to enter pediatric practice in Baltimore in the early 1920s. Until the time he accepted a full-time academic position in 1946, Dr. Wilkins had practiced pediatrics for 25 years with intense intellectual curiosity and great compassion for his patients. This author has on several occasions in the past met adults in Baltimore who remembered Dr. Wilkins fondly as their pediatrician. These individuals had no idea that Dr. Wilkins had made major contributions to medicine as an endocrinologist and a geneticist.

In 1935, Dr. Edwards Park, who was instrumental in the development of various subspecialties in pediatrics, invited Lawson Wilkins to establish an endocrine clinic in the Harriet Lane Home of the Johns Hopkins Hospital. Dr. Wilkins was reluctant since endocrinology at that time was the trade of quacks and charlatans. He accepted the position, however, and with Drs. Fuller Albright, John Eager Howard, George Thorn, Robert Williams, and a few others, he transformed endocrinology into a respectable subspecialty.

Wilkins focused on the problems in pediatric endocrinology - particularly problems of growth and genetics - while his confreres tended to the accumulation of knowledge about endocrinology in adults. Although he was intensely interested in the metabolism and control of carbohydrate and fat metabolism, he assiduously avoided a clinical interest in diabetes. Possibly this was because Dr. Harriet Guild of the Harriet Lane staff had established a diabetes clinic and, characteristically, Dr. Wilkins would not intrude on the work of others unless invited. Interestingly, he never considered diabetes a disease of the endocrine system, although he believed hypoglycemia was.

Lawson Wilkins was more than a scientific giant. He was a man of great magnetism and personality. Few who knew him could forget his bass voice which he put to good use singing ballads and bawdy songs long into the night. He loved to sail his boat on the Chesapeake Bay and tell jokes, which he masterfully embellished. He also adored - and was adored by - Lucile Mahool, his first wife, and Teence Anderson, to whom he was married after Lucile died in 1959.

At a meeting in Baltimore of the Lawson Wilkins Pediatric Endocrine Society in the mid-1960s, Dr. John Eager Howard* related the following about Dr. Wilkins: "When I first met Wilkins, which was at a time I had heard about his studies that Dr. Park exalted, I was even more impressed by the vitality of the man than by his scientific studies. In response to my knock on the door, the rafters fairly reverberated to the booming voice that urged us to come in. His whispers in a conference could cause consternation, for his 'That fellow is putting out pure hogwash' might have been heard all over the room. But I should hasten to say that his comments were rarely uncomplimentary, for an immense generosity toward others was one of his most endearing qualities." In accord with Dr. Howard's observations, this author found Dr. Wilkins to be a paradox in that he was gruff but gentle. And while he always dominated the situation, he never exhibited dominating behavior toward individuals.

Another mark of the quality of Dr. Wilkins' personality was the grace with which he relinquished his pediatric endocrine clinic and training program to Dr. Claude Migeon and this author in 1960. During the next three years, before he died in 1963, he was present much of the time, he remained intellectually curious, and he continued to contribute in all respects.

SCIENTIFIC CONTRIBUTIONS

Lawson Wilkins greatly expanded our knowledge of endocrine physiology and pathophysiology. Some of us have been fortunate enough to have shared in his experiences in establishing pediatric endocrinology as a subspecialty. Drs. Albert Bongiovanni,* Claude Migeon, and Walter Eberlein shared his interest in adrenal steroid metabolism and the pathophysiology produced by deficiencies of various enzymes for cortisol synthesis, including defects in 21 hydroxylation and 11 hydroxylation that produce congenital virilizing adrenal hyperplasia. In 1950, Drs. John Crigler, Robert Klein, Lytt Gardner,* Claude Migeon, and Eugenia Rosemberg joined Dr. Wilkins in successfully treating the first patients with congenital virilizing adrenal hyperplasia with cortisone. As always, Dr. Wilkins applied the knowledge he gained from his physiologic studies to therapy.

(*Deceased)

Drs. Melvin Grumbach and Judson Van Wyk worked with Dr. Wilkins in his studies of sexual differentiation. In this area, Dr. Wilkins applied what had been learned from the animal experiments of Alfred Jost to postulate and prove that the anatomy in gonadal agenesis and pseudohermaphroditism in human beings could be explained by the presence or absence of androgens and Mullerian inhibiting factor.

It was with Dr. Wilkins that Lytt Gardner* developed his interest in genetics and cytogenetics. It was Dr. Wilkins and his students who were among the first to apply the cytological techniques of Dr. Murray Barr to identify the inactivated X chromosomes (Barr bodies) in the nuclei of patients with Klinefelter's syndrome and in female pseudohermaphrodites. These diagnostic aids facilitated the diagnosis and therapy of patients with abnormalities of sexual development.

With Dr. Wilkins, Dr. George Clayton demonstrated that enzyme defects in the synthesis of thyroid hormone metabolism produce pathologic changes in the thyroid that simulate thyroid carcinoma. Dr. Wilkins had previously demonstrated during his years in practice the effect of thyroid hormone on cholesterol and creatinine metabolism.

Dr. David Smith* and this author benefitted from Dr. Wilkins' astute record keeping; he was a master in maintaining growth charts and other documents. With him, we published the effect of thyroxin treatment on the mental development of cretins.

These were classic physiologic studies in which the effects of a hormone were investigated clinically. He had demonstrated during this same period that the epiphyses in patients with thyroid deficiency were misshapen as they calcified (epiphyseal dysgenesis) and delayed in appearance, and that epiphyseal dysgenesis was a frequent finding in the untreated cretin. With treatment, the epiphyses that had not appeared because of thyroid hormone deficiency were often dysgenetic when they did appear, but the epiphyses that were expected to appear following the chronologic age that treatment was begun were always intact in their development.

THE SECOND GENERATION AND BEYOND

Other pediatric endocrinologists from the United States who trained with Dr. Wilkins between 1946 and 1960 were Drs. Thomas Shepard, Gerald Holman, José Cara,* David Mosier, William Cleveland, Ralph David, Orville Green, Malcolm Martin, Samuel Silverman, and Robert Stempfel. Many students from abroad who are now professors also trained with Dr. Wilkins. These include Drs. Jean Bertrand, John Eckert, John Gerrard, Casaer Bergada, Thedorus Papadatos,* and Andrea Prader* who followed in Lawson's image as a major founder of pediatric endocrinology in Europe, and Henning Anderson.* These endocrinologists and professors have trained the third generation of pediatric endocrinologists who in turn have trained the fourth generation.

Dr. Wilkins wanted to be called "Lawson" by "his boys" as he called those who trained under him, but esteem for him was so great that he remained "Dr. Wilkins" to most for many years.

It is not by chance, however, that there was only one female fellow, Dr. Eugenia Rosemberg, prior to 1960. It was simply Dr. Wilkins' policy not to accept women as fellows. He respected the intellect of female physicians, but he was reluctant to let them examine the male teenagers who came to him for consultation. With the acceptance of Drs. JoAnne Brasel, Virginia Weldon, and Irene Solomon as pediatric endocrine fellows at Johns Hopkins in the early 1960s (when he was professor emeritus but still active), he relented and realized that he had been unduly restrictive.

We in pediatric endocrinology, pediatrics, and genetics are indeed blessed to have had such a man to lead us. The history of Lawson Wilkins is well worth passing along to the third and fourth generations of pediatric endocrinologists, and it is to be hoped that they will pass it along to the fellows who train with them.

(*Deceased)

REFERENCES AND THEIR HIGHLIGHTS

1. Wilkins L. Presidential Address to American Pediatric Society. *Am J Dis Child* 1962:104:449-456.

Dr. Wilkins wished to chastise pharmaceutical firms for their focus on the commerce of manufacturing and marketing drugs and to warn physicians to avoid the pitfalls of over prescribing medications and/or prescribing the newest medicine in the pipeline when its efficacy and the potential long-term toxicity are obscure. This masterful presentation was both educating and chastising. The following capsulizes Wilkins' closure: (1) Remember the Oath of Hippocrates, (2) Give no drug if it is not needed. Placebos rarely have a place in pediatrics, (3) Remember that practically every effective drug has potentials for toxic side-effects. (4) Neither discuss nor prescribe drugs by brand name, (5) Never use a drug or mixture without full knowledge of its chemical nature and pharmacological action. (6) Do not attempt to learn your new therapeutics from the trade brochures or even the PDR, (7) Do not hasten to use the 400+ new drugs coming on the market each [illegible]

particularly if they are variants of drugs with which you already have had experience, (8) Wait, wait, wait - and then wait. Let the other fellow poison his patients.

2. Bongiovanni AM. Presentation of the John Howland Medal and Award of the American Pediatric Society to Dr. Lawson Wilkins. *J Pediatr* 1963:63:803-807.

Dr. Bongiovanni pays tribute to Lawson Wilkins for all of his accomplishments with the help of Wilkins only sibling and records: "He had a child like curiosity and spirit of inquiry that kept him young. He was never struck with the prejudices of a prior era. His advantages were scholarly acquaintance with earlier discoveries, an intimate knowledge of clinical aspects, and a firm hold on the basic sciences. His multiple interests are reflected in the diversity of titles to his innumerable publications, which include studies on serum potassium, ulcers of the tongue, rickets, immunization against dysentery, meningitis, pyuria, epilepsy and many diverse aspects of endocrinology." The presentation in this reference was a remarkably successful rendering of insight about the personality and personal characteristics of Lawson Wilkins.

3. Wilkins L. Acceptance of the Howland Award. *J Pediatr* 1963:63:809-811.

Dr. Wilkins paid extensive gratitude to his mentors and colleagues, including fellows, which reflected his true sincerity for his colleagues' contributions and collaborations, and to educate his listeners. As he stated, "I wish to take the privileged opportunity to emphasize the importance of the clinician and clinical investigator in contributing to basic and fundamental knowledge." His views about clinical investigation in abbreviated wording was as follows: It is the clinician who must seek out and bring to attention the human experiments of nature . . . no one can reproduce in the laboratory most of the inborn enzymatic defects

. . . I always permitted my assistants to delve into any type of problem which interested them . . . The scientist must have an insatiable curiosity to seek knowledge along any lines

. . . The clinical investigator must have curiosity and, if he has such curiosity, nearly every patient he sees will call forth many questions of real importance which have never been answered. The clinical investigator will be impelled to attempt to answer these questions by studies upon the patient.

4. Wilkins L. The Evolution of Endocrine Diagnosis and Treatment: The Addison Lecture. *Guys Hospital Gazette* 1954:March 19th, pages 1-9.

Dr. Wilkins gave a masterful presentation of the history of clinical endocrinology beginning with Graves' classical description of thyrotoxicosis in 1834 and a current (1954) discussion of the interrelationships of the endocrine glands and their hormones including diagnostic methodology available, differentiation of CAH in males from other types of sexual precocity, diagnosis of sexual infantilism, etc. The result was a very erudite lecture revealing how successful Dr. Wilkins was in sorting out the diagnoses and treatment of various pediatric endocrinopathies. The content of this lecture was incorporated into the 2nd Edition of his textbook, The Diagnosis and Treatment of Endocrine Disorders in Adolescence and Childhood (1957).

5. Blizzard RM. Pediatric Profiles: Lawson Wilkins (1894-1963). *J Pediatr* 1998:133:577-580.

Dr. Blizzard was invited to write such a profile as the *Journal of Pediatrics* was composing a series on the profiles of those who had pioneered in the specialty of pediatrics. His initial goal was to introduce an unusual story to the readers of his first encounter with Lawson Wilkins. This unusual encounter characterized Wilkins' personality - honesty, directness, a no nonsense approach, leadership, preciseness, and the expectation that one hearing a private conversation would keep the confidence of the discussants. The paper also describes in subsections The Wilkins personality, Wilkins as a physician, Wilkins as an investigator, and Wilkins as a teacher. The article ends with brief descriptions of his last years and conclusions.

6. Bongiovanni AM, et al. To Honor Lawson Wilkins, MD in His 65th Year. *J Pediatr* 1960:57:317-325.

Dr. Bongiovanni provides a personal accounting given by Dr. Edwards A. Park (pages 317-322) of his professional relationships with Lawson Wilkins and accountings of personal relationships with Lawson Wilkins by some of his colleagues of the early historic days, including Douglas Hubble of Scotland. The accountings of Hubble and Park are particularly insightful and should be read by those wishing to more completely understand Dr. Wilkins as a clinical investigator and as a unique personality.

7. Money J. Foreword to the 3rd Edition of *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*. By Lawson Wilkins with the editorial assistance of Robert M. Blizzard and Claude J. Migeon; 1965:pages vii-xi.

Dr. Money wrote this foreword after Lawson Wilkins' death with the primary objective of recording Dr. Wilkins' professional and personal characteristics by one who had worked closely with him for more than a decade.

Dr. Money delivered a very thorough and appropriately lengthy personal and professional history of Dr. Wilkins. Dr. Money's closing paragraph is particularly pertinent as it is conceptually flattering and truthfully accurate: "Lawson Wilkins achieved fame, but as a by-product of accomplishment. His life's goal had been to achieve, not to become famous."

8. Fisher DA. A Short History of Pediatric Endocrinology in North America. *J Pediatr* 2003 (In preparation).

The purpose of this article is to record for posterity a historical perspective of the founding and development of pediatric endocrinology as a subspecialty, of the Lawson Wilkins Pediatric Endocrine Society, of pediatric endocrine training programs, of pediatric diabetes as a discipline, and of advances in understanding, diagnosing, and treating pediatric endocrinopathies since 1950. A very excellent and complete presentation of the topic has been written by Dr. Fisher. As part of this, Lawson Wilkins' major roles as pediatrician, founder of the subspecialty, clinical investigator, and academician are evident.

9. Migeon CJ. *The Origins and Establishment of the LWPES*. http//www.lpwes.org/history.html. The concept and history created by Lawson Wilkins invitation in 1963 of a scientific gathering to the formal creation in 1972 of a Society is interestingly detailed.

Abstracts from the Literature

Body Mass Index and Segmental Proportion in Children with Different Subtypes of Psychosocial Short Stature

Psychosocial short stature (PSS) has been classified by the authors into 3 categories: (1) Type IIA are hyperphagic children, in whom there is reversible growth hormone (GH) insufficiency with rapid catch-up growth with a change in their living environment but with minimal response to exogenous GH; (2)Type IIB is a heterogeneous sub group of non-hyperphagic children who have normal GH secretory dynamics and minimal or absent increase in growth rate with change in their environment and variable response to GH; and (3) Type III are children with anorexic eating habits, with an onset as early as infancy, with failure to thrive, depression, normal GH secretory dynamics, and significant growth response to exogenous growth hormone. Gohike et al report anthropometric evaluations of 46 children with PSS, before and after change in their environment (Table 1).

Significant improvement in height velocity SDS after intervention was observed in all groups. ANOVA failed to show any significant differences in growth velocity between groups. There was no significant change with treatment in body proportion in type IIA (hyperphagic) or in type IIB (heterogenous) children. In type III (anorexic) children, the body proportions decreased significantly after intervention indicating relatively shorter upper segments after treatment. In those who received GH treatment (n = 21), there was no significant change in body proportion after GH therapy. Body Mass Index

Table 1

Clinical Data of 46 Children with PSS

Classification	**Type IIA (n = 20)**	**Type IIB (n = 16)**	**Type III (n = 10)**
Mean age at presentation (years)	8.6	7.6	10.2
Age range (years)	4.9-15	3.8-14.9	5.4-14.9
Sex	9F. 11M	4F. 12M	6F. 4M
IUGR	2F. 2M	0F. 5M	2F. 2M
Mean bone age delay at presentation (years) (SD)	1.69 (1.0)	1.69 (1.3)	2.0 (1.5)
Prepubertal at presentation	18	15	9
Type of intervention			
Social services only	17	5	3
Social services and GH therapy	3	11	7

Adapted from: Gohlke BC, et al. *Eur J Pediatr* (220) 161: 250-254.

(BMI) did not increase in any of the groups after intervention and there were no significant changes in bone age. Multiple regression analysis showed that the type of PSS was a predictor for height velocity after intervention. The greatest effect in removal from adverse home events were in the type IIA (hyperphagic) subjects. The authors state that their findings should be helpful to clinicians managing children with PSS because of the emphasis on appetite disturbance and the variable treatment responses.

Gohlke BC, et al. *Eur J Pediatr* 2002;161:250-254.

***First Editor's Comment**: PSS, first described in 1947 by Talbot et al,[1] is often difficult to diagnose. Variable GH secretory dynamics, and responses to exogenous GH therapy make it important to attempt to better understand the etiologies involved and their potential response to psychosocial changes. The current manuscript report data on a large number of subjects with PSS and suggested that BMI is not useful in predicting response to treatment, but that categorization based on appetite may be of use in predicting growth changes. It is unfortunate that their data were not analyzed separately for those with intrauterine growth retardation (IUGR) and for those with and without GH insufficiency. However, the heterogeneous composition and variable treatment of these children strengthen the conclusions based on categorization of subjects by their eating behavior.*

Reference

1. Talbot NB, et al. *N Engl J Med* 1947;236:783-789.

William L. Clarke, MD

***Second Editor's Comment**: PSS should be considered by pediatric endocrinologists or pediatricians in the differential diagnosis of short stature when a short child is seen in the clinic. If the possibility of this diagnosis is not considered and explored in the history, the diagnosis will be missed. PSS occurs much more frequently than is realized. Many parents of children with PSS (particularly Type II A of the English classification) are not concerned about their child's stature because the parents are psychologically rejecting the child.*

PSS is a spectrum of entities as Gohlke, Frazer, and Stanhope state. The classification is muddy for this reason. In the patients reported by Gohlke et al there was no child less than 3.8 years of age. In the classification listed in Lifshitz's Pediatric Endocrine Text[1] (3rd Edition, 1996), infants with PSS comprise a broad clinical spectrum. This should be kept in mind so that the diagnosis of PSS is made and treatment properly instituted, which in my opinion is not GH, but removal of the child from the adverse environment, particularly for type II.

Types of PSS as Described in the 3rd Edition of Pediatric Endocrinology[1]

At least three subtypes of psychosocial short stature have been recognized (Table 2). The first (type I) occurs in infants and children 2 years of age or younger. These infants usually have failure to thrive (nutritional deficiency), as well as short stature, and have been very adequately described by Krieger, Whitten, and colleagues.[2-6] There is no evidence that these children have a hormonal disturbance, such as growth hormone deficiency, and they usually recover when sufficient calories are ingested. Their parents do not usually blatantly reject the child. The mothers characteristically have multiple children or responsibilities. They are usually disorganized, and the children do not receive the food or the attention they need, but the attention they receive is usually adequate for infants to again grow, if they are given adequate nourishment. Nevertheless, growth in some may be inadequate without further psychosocial interventions, as reported by Bithoney et al.[7-9]

Type II PSS has been called transient hypopituitarism, reversible hyposomatotropism, emotional deprivation, maternal deprivation, psychosomatic dwarfism, abuse dwarfism, and the "garbage can" syndrome. The term PSS is preferable to definitions that include the presence or absence of GH, the presence or absence of overt psychologic abuse, or emotional deprivation. This type occurs characteristically in children 3 years of age and older.

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Table 2

Characteristics of Various PSS Syndromes[1]

Type	Age of Onset	Failure to Thrive	Bizarre Behavior	Depression	GH Secretion	Parental Rejection	GH Responsiveness
I	Infancy	Usually	No	Often	Normal	No (see text)	?
II	≥3 years	Some & some overweight	Usual	Very often	Decreased or absent often	Usual	Minimal at doses used
III	Infancy or later	Not usual	Not usual	Yes	Normal	Concern, not rejection	Significant at dose used

Adapted from: Blizzard RM, Bulatovic A. In: Lifshitz F, ed. *Pediatric Endocrinology 3rd Edition*. New York: Marcel Dekker, 1996:83-93.

There is a greater psychologic component, and GH response may be inadequate after stimulation with pharmacologic agents, such as arginine or insulin. Other abnormalities indicating adrenocorticotropic hormone (ACTH), thyroid stimulating hormone, and gonadotropin deficiency may be noted; however, GH deficiency is the most common endocrine aberrancy. The parents in this group usually reject their children and abuse them psychologically. The fathers and/or mothers are frequently chronic alcoholics. Occasionally type I patients are observed to advance into type II, which is not surprising.

Type III of PSS was described by Boulton et al,[10] who studied seven children aged 3.6 – 11.6 years who did not have the bizarre signs and symptoms of type II patients. They were significantly depressed and/or had a disorder of attachment often dating from infancy. In contrast to previously reported patients they secreted GH when tested and had a significant increase in growth when given growth hormone treatment. A lesser response was obtained with a placebo. The authors emphasized that type III PSS patients did not show lack of discrimination in relationships, nor did they display the self-destructive behavior, pain agnosia, or bizarre eating and sleeping disorders seen in many type II patients. In addition, the parents were not indifferent and rejecting, as are those with PSS type II. The parents also had insight into the problem, which was not characteristic of the parents of other patients with PSS and several felt guilty and/or had depression.

The classifications discussed here by the English group and that presented in Lifshitz's Endocrine Text are compatible. Type I, as described above, should remain as type I and be applied to infants and very young children. Type II pertains to children with severe PSS of the hyperphagia type. In my opinion, type II should be limited to this group. Type III is where further subclassifications should be placed. For example, type IIIA could (should) be the group described by Boulton[10] and type IIIB of the type referred to by Gohlke et al. With this classification type IIIA & B can be subdivided or a type IV added as further subgroups are recognized. I wonder if Drs. Gohlke et al or others agree with my thinking? A letter to the Editors of GGH will be most welcome.

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Robert M. Blizzard, MD

Leanness, Extended Lifespan & IGF-1 Receptor Mutations in Mice: Fascinating Observations

In flies and worms, loss-of-function mutations in insulin and insulin-related cell signaling pathways have led to *increase* in life span of the species studied. In order to evaluate these pathways in a mammalian specie, the present investigators developed mice with hemizygous loss of one insulin-like growth factor-1 receptor (IGF-1R) allele and studied their longevity. The hemizygous IGF-$1^{+/-}$ mice were generated by deletion of exon 3 of

the gene encoding IGF-1R; these mice had 50% of the IGF-1R levels that intact animals had. Homozygous inactivation of the gene encoding the IGF-1R (IGF-1R$^{-/-}$) was lethal. During nursing, IGF-1R$^{+/-}$ and intact (IGF-1R$^{+/+}$) mice grew identically; after weaning there was a slight decrease in growth (-6% to -8%) in hemizygous mice relative to intact animals through 11 weeks of age. IGF-1R$^{+/-}$ female mice lived 33% longer and males 16% longer than did IGF-1R$^{+/+}$ mice, and female hemizygous mice outlived their male counterparts. (Figure) As anticipated, serum IGF-1 concentrations were higher in IGF-1R$^{+/-}$ mice than with control animals, while insulin levels were normal. Glucose tolerance was impaired in IGF-1R$^{+/-}$ male but not female mice. Energy balance in mutant and control animals was similar in food intake, body temperature, physical activity, metabolic rate and fertility. The ability to withstand an oxidative stress was greater in mutant than control animals both *in vivo* and *vitro*. In cultured fibroblasts, the amounts of several signal transduction molecules downstream of the IGF-1R were decreased relative to the activity of control fibroblasts. In particular, levels of phosphorylated p66 shc, an activator of mitogen activated protein (MAP) kinase, were reduced by one-half, suggesting that perhaps a decrease in the rate of cell division might be an important factor in increasing longevity. The investigators conclude that in mice the partial inhibition of IGF-1 signaling leads to increase in life span.

Bluher et al demonstrated that in mice in which there has been localized "knock out" of fat specific insulin-receptors (FIRKO) (in contrast to generalized loss of IRS which leads to insulin resistance, diabetes mellitus, and obesity), there was extension of life span despite normal caloric intake and without clinical or biochemical abnormalities. FIRKO mice were approximately 20% lighter and their body fat content approximately 60% lower than control animals, despite eating similar quantities of food. Control animals lived an average of 753 days, while FIRKO mice lived 887 days (+134 days, +18%); median life span in FIRKO was increased by +3.5 months and maximum life span by +5 months. Fertility of the FIRKO mice was not reported. The investigators concluded that low body fat content (leanness) rather than decreased food intake was the primary factor contributing to increase in life span of the FIRKO mice.

Holzenberger M, et al. *Nature* 2003;421:182-186.

Bluher M, et al. *Science* 2003;299:572-574.

Editor's Comment: *There is increasing evidence that insulin, growth hormone (GH), and IGF-1 are intimately involved with the duration of life. Experimentally, partial caloric deprivation increases life span while decreasing serum concentrations of IGF-1. Mice with GH deficiency (GHD) such as Ames (Prop$^{df/df}$) and Snell (Pit1$^{dw/dw}$) mice are extremely long-lived albeit dwarfed and infertile, as are mice in which the GH receptor has been "knocked-out."*

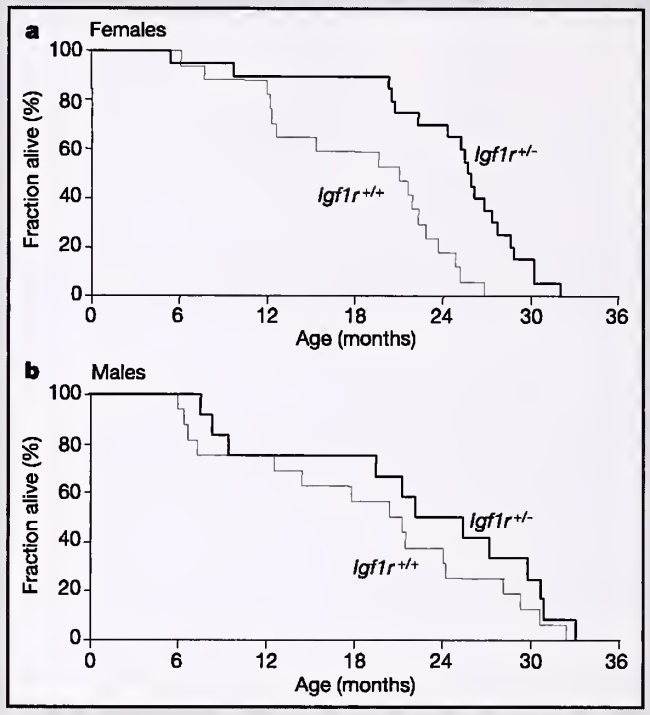
The manuscripts present several interesting observations in addition to those on longevity. Thus, partial inactivation of the IGF-1R gene led to slightly subnormal growth in mice, suggesting that variants of this gene might play a role in the diversity of height in man. Also of interest were the gender specific effects of partial loss of IGF-1R which was more pronounced in females than males which indicated that sex-specific factors may modulate the effects of IGF-1R.

While it is not possible to transpose these data to man, they make one wonder whether we may be adversely affecting life span by treating our GHD adult patients with rhGH. Perhaps it might be less risky to treat the cardiovascular and skeletal abnormalities of the adult with GHD with agents other than rhGH.

Allen W. Root, MD

Figure

Lifespan extension in *Igf1r*$^{+/-}$ mice with respect to *Igf1*$^{+/-}$ (WT) mice



a - *Igf1r*$^{+/-}$ females (thick line) live a mean of 33% longer than their wild-type littermates (756 ± 46 compared with 568 ± 49 days; $P < 0.01$, *t*-test). Kaplan-Meier analysis of survival revealed a later decline in *Igf1r*$^{+/-}$ mice compared with wild type ($P < 0.001$, Cox's test). **b -** *Igf1r*$^{+/-}$ males live 15.9% longer than wild-type littermates (679 ± 80 compared with 585 ± 69 days; NS).

Reprint with permission from: Holzenberger M, et al. *Nature* 2003;421:182-186.

Hypothalamic Insulin Signaling is Required for Inhibition of Glucose Production

Insulin has many energy modulating actions that take place in the hypothalamus, such as inhibition of feeding. The investigators studied the effects of infusing insulin, an insulin mimetic, and inhibitors of insulin action. Infusion was done in the intra-third cerebral ventricle (ICV). Hepatic glucose production and peripheral glucose consumption were determined. Steady state of serum insulin concentrations were achieved by using systemic pancreatic-insulin clamps.

ICV infusion of insulin/insulin mimetic at basal insulin concentrations led to a 7-fold increase in glucose infusion rate to maintain euglycemia. Thus, ICV glucose enhanced peripheral insulin action. Employing radiolabeled glucose and kinetic glucose studies, the investigators demonstrated that ICV insulin decreased the rate of hepatic glucose production by 40+% while not altering peripheral glucose consumption. Inhibition of insulin action in the hypothalamus by co-infusion of insulin antibodies or an antisense disrupter of insulin receptor synthesis antagonized the effect of insulin on glucose production. Further studies demonstrated that the intracellular mechanism(s) through which hypothalamic insulin exerted its effect on glucose production involved the phosphoinositide-3-kinase signal transduction pathway and ATP sensitive potassium channels. However, the manner in which hypothalamic insulin impaired hepatic glucose production was not identified by these studies. The authors suggest that hypothalamic insulin (as well as other factors such as leptin and melanocortins) may monitor and modulate exogenous energy intake relative to endogenous energy consumption. Failure of hypothalamic insulin function may lead to peripheral insulin resistance and may be a factor in the pathogenesis of the dysmetabolic syndrome and type 2 diabetes mellitus.

Obici S, et al. *Nature Med* 2002;8:1376-1382.

***Editor's Comment**: The physiological importance of insulin action within the central nervous system is well described in the content of this manuscript. The demonstrations reported open yet another site at which a metabolic error may lead to clinical illness. It is crucial to determine the specific mechanisms by which the hypothalamic action of insulin is recognized at the hepatic level and to develop a method(s) by which one may assess hypothalamic insulin function in the intact human.*

Allen W. Root, MD

Hyperzincaemia and Hypercalprotectinaemia: A New Disorder of Zinc Metabolism

The authors describe five patients (including a mother and her son) who had a multidimensional illness comprised of recurrent infections, rash, arthritis/ vasculitis, hepatosplenomegaly, and growth retardation in infancy and childhood. Although these findings were consistent with zinc deficiency, the patients had marked hyperzincemia due to its binding to greatly elevated amounts of a zinc-binding protein called calprotectin. Calprotectin is a calcium and zinc binding protein complex of two S100 plasma proteins termed S100A8 and S100A9 (also termed proteins MRP8 and MRP14, respectively). It is present in the cytosol of phagocytes and is released into plasma as phagocytic neutrophils are destroyed. In these patients, plasma zinc concentrations were 5-10 times higher than the upper normal range (18 µmol/L), while calprotectin concentrations were 1000 fold greater than the upper normal value (850 µg/L), suggesting that free plasma zinc concentrations were likely to be low. Individual patients were anemic, thrombocytopenic, and had low numbers of monocytes and B lymphocytes. Chromatographic analysis of S100A8 and S100A9 proteins was normal, suggesting no major mutations or post-translational modifications of calprotectin. Since there was no evidence of increased neutrophil turnover rate, the investigators hypothesized: (1) that the increased plasma concentrations of calprotectin reflected its decreased rate of degradation; (2) that the patients were zinc deficient because of the high affinity of calprotectin for zinc; and (3) that calprotectin itself may have been cytotoxic to neutrophils and other tissues.

Sampson B, et al. *Lancet* 2002;360:1742-1745.

***First Editor's Comment**: The new syndrome comprises patients with an apparent "functional zinc deficiency" despite high plasma concentrations of this element. Although "free zinc" concentrations were not measured, they were thought to be low. In addition, the authors did not report the effects of a trial of therapy with supplemental zinc in these subjects. Thus, it*

Table

Clinical and laboratory data of patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	18	9	14	35	21
Sex	M	F	M	F	M
Growth failure	<3rd percentile	<3rd percentile	<3rd percentile	Normal	Normal
Hepatosplenomegaly	Yes	Yes	Yes	Yes	Yes
Dermatological symptoms	Vasculitis	None	None	Vasculitis, eczema	Vasculitis, furuncules ulcers
Rheumatic symptoms	Arthritis	Arthritis	Arthritis	Arthritis, uveitis	Arthritis
Plasma C-reactive protein (mg/L)†	41-143	100-200	22	17	45-146
Haemoglobin (g/L)	80	90	109	125	80
Total white-cell counts (10^9 cells/mL)	2-0	3.7-5.0	1.5	5.0	3.8
Monocytes	0		1.9%	1.9%	4.3%
Plasma zinc (mol/L)‡	180-200	82-96	160-200	175	77
Plasma calprotectin (g/L)§	6.5	1.4, 2.55	9	6.1	1.5

†Reference <10 mg/L. ‡Reference 10-18 mol/L. §Reference <1 mg/L

Adapted from Sampson B, et al. *Lancet* 2002;360:1742-1745.

hypothesis regarding "functional zinc deficiency" remains unproven. Since calprotectin is a calcium binding protein, it would have been of interest to report total and ionized calcium values in these patients.

Zinc deficiency may be congenital or acquired. Acrodermatitis enteropathica (OMIM 201100) is an autosomal recessively transmitted disease characterized by bullous lesions of the skin, alopecia, diarrhea, and growth failure with hypozincemia. Administration of supplemental zinc ameliorates these abnormalities. Approximately 50% of patients with acrodermatitis enteropathica have a loss-of-function nonsense or missense mutation in SLC394A (Solute Carrier Family 39 [Zinc Transporter], Member 4) encoding a renal- and intestine-specific transmembrane zinc transporter protein (OMIM 607059, chromosome 8q24.3). Zinc deficiency may be acquired due to dietary deficiency, decreased absorption due to co-ingestion of zinc-binding materials such as clay or phytates, malabsorption as in patients with chronic inflammatory bowel disease, or excessive excretion as in patients with sickle cell disease and hyperzincuria.

Allen W. Root, MD

Second Editor's Comment: *I am puzzled by the possibility of copper deficiency in these patients. The clinical picture and the anemia and leucopenia are typical of it. A deficit of this mineral would likely result in a deficiency of Ca/Zn SOD (super oxidase desmutase) though I do not know of studies of its effects on calprotectin.*

Fima Lifshitz, MD

Initial Treatment Dose of L-Thyroxine in Congenital Hypothyroidism

The American Academy of Pediatrics (AAP) recommends an initial L-thyroxine dose of 10 to 15 mcg/kg/d for the treatment of congenital hypothyroidism (CH). Several studies have shown that early high dose therapy which quickly produces serum T-4 levels within the "normal" neonatal range may be associated with the

development of near normal IQ scores; whereas therapy with lower dosages are associated with a delay in achieving normal T-4 concentrations by as little as 1 week may result in lower IQ scores. Thus, pediatricians and pediatric endocrinologists need to be familiar with treatment regimes that achieve the T-4 goal with as little delay as possible, yet do not produce untoward side effects such as craniosynostosis.

Selva and colleagues present data obtained in 47 congenitally hypothyroid neonates (BW 3-4kg) using a prospective randomized study of 3 different L-thyroxine dosing regimens (Group 1 – 37.5mcg/d; Group 2-loading dose 62.5mcg/d x 3d, then 37.5mcg/d; Group 3 – 50mcg/d). Serum T-4, free T-4, T-3, free T-3, and TSH were measured at baseline, 3 days, and 1, 2, 4, 8, and 12 weeks after starting treatment. No changes in treatment dose were made for 2 weeks. At that time, dosages were altered using the following important algorithm to maintain serum T–4 concentrations between 10 – 15 mcg/dL; a)T-4 < 8.5mcg/dL, increase dose by 12.5mcg/d, b) T-4 between 8.5 and 9.9mcg/dL, increase dose by 6.25mcg/d, c) T-4 between 15.1 and 16.5mcg/dL, decrease dose by 6.25mcg/d, d) T-4 greater than 16.5mcg/dL, decrease dose by 12.5mcg/d.

Pre-treatment thyroid levels were similar in all three groups. Infants in Groups 2 and 3 achieved target T-4 levels by 3 days, while infants in Group 1 did so by 1 week of age. Subjects in Group 3 had T-4 levels above 16mcg/dL by 1 week, while the others were in the target range at both 1 and 2 weeks. TSH remained elevated in Groups 1 and 2 for the first 2 weeks. After 2 weeks, serum T-4 remained within the target range in all three groups, but doses were adjusted as outlined above. At 12 weeks, mean L-thyroxine dose was 36.7 mcg/d (approximately 6mcg/kg/d) in all groups, which was associated with ideal target levels of T4, T3, and TSH. Free T-4 levels rose above normal by 1 week and remained above normal at 12 weeks in all age groups. There were no significant differences in TSH concentrations at 12 weeks among the groups.

When patients were divided into severe and moderate CH categories based on serum T-4 above or below the median value, the differences in initial T-4 levels were abolished by 3 days for Group 3 infants and by 1 week for the others.

The authors state that their data shows that a loading dose of 62.5mcg/d x3 days followed by a dose of 37.5mcg/d raises serum T-4 levels quickly but does not normalize TSH levels. However, the sustained dose of L-thyroxine (50mcg/d – Group 3) normalized TSH levels within 2 weeks and abolished any difference in serum T-4 levels between severe and moderate CH infants by 3 days. Consequently, they recommend the use of a higher target range of 10 to 18mcg/dL for T-4 for the first 2 weeks of therapy to insure that the benefits of therapy are maximized.

Selva K, et al. *J Pediatr* 2002:141:786-792.

***Editor's Comment**: It may seem surprising to read a paper dealing with the "correct" L-thyroxine dose for treating infants with CH, when most neonatal screening programs have been in place for approximately 20 years and have been highly successful in identifying these infants and seeing that they receive what has been considered "appropriate" treatment. However, the medical community, despite well-delineated guidelines from the AAP, has yet to define "appropriate" treatment. The article by Selva et al helps clarify three different treatment regimens. They are to be commended for the prospective randomized protocol followed. It is interesting that they refrain from "recommending" a single or favorite regimen. Indeed all three regimens work well if the goal is to normalize serum T-4 within 1 week. Quicker attainment of the target range requires a loading dose for three days. The accompanying algorithm for adjusting L-thyroxine doses is helpful and all of these data and recommendations need to be disseminated to those caring for neonates.*

William L. Clarke, MD

***Second Editor's Comment**: This detailed analytical study is accompanied by a detailed analytical report pointing out that several groups have demonstrated as much as a 20 point IQ deficit in severely affected CH infants who did not have rapid and complete conversion of serum hormonal levels of T4, T3, free T4 and T3, and TSH to normal. The article convinced me that a treatment protocol as used for group 3 is currently the best available.*

In an accompanying editorial by Dr. Nancy Hopwood[1] of the University of Michigan, emphasis is given to the importance of using only tablets of T4 because liquid preparations may be unreliable. She also points out that persistent TSH elevation can result from faulty absorption of T4 in patients with milk allergy, malabsorption of various causes, with soy formulas, iron therapy, and with acidic juices in children of all ages. The article by Selva et al and the editorial by Dr. Hopwood fit together splendidly.

Reference

1. Hopwood NJ. J Pediatr 2002;141:752-4.

Robert M. Blizzard, MD

Survival Profile for Down Syndrome

Down syndrome is the most common form of inherited intellectual disability. In addition, it is associated with growth deficiency, hypotonia, characteristic craniofacial appearance and developmental anomalies involving the heart and other organ systems. Survival of these patients has changed dramatically over the last several decades primarily because of surgical intervention for cardiac defects. For example, life expectancy increased from 12 years in England in 1949 to recent estimates of over 50 years in western countries. These estimates are based on cross-sectional data because there is little longitudinal information available. Moreover, it is known that adults with Down syndrome are predisposed to a number of disorders including obesity, hypothyroidism, epilepsy, dementia, and Alzheimer's disease; however the impact of these disorders on survival is unknown.

To define the survival profile for those with Down syndrome, Glasson and colleagues assessed survival in 1,332 patients (45% female) born between 1902 and 2000, mostly in Australia. Most patients had had standardized intelligence testing. Death had occurred in 20%. Kaplan-Meier survival probabilities were calculated separately for sex, level of intellectual disability and decade of birth.

The analysis showed that the overall life expectancy for patients with Down syndrome approaches that of the general population in Australia. Seventy-five percent of cases had survived to 50.0 years, 50% to 58.6 years and 25% to 62.9 years of age. The mean life expectancy for males was greater than females by 3.3 years with the median survival probabilities of 61.1 for males and 57.8 for females. The difference was attributed to a higher incidence of heart defects in females. When examined by decade born, each successive birth group showed increased survival consistent with progressive improvement in medical care. No association was found between level of intellectual disability and survival, which was surprising to the authors because an association had apparently been found in an earlier study.

Approximately 25% of all Down syndrome deaths occurred between the ages of 58 and 63 years. No clear explanation for this was found nor is there any certainty that the trend will continue in patients born more recently. The authors raise the possibility that it could reflect mortality associated with the above mentioned chronic diseases to which adults with Down syndrome are predisposed.

Glasson EJ et al. *Clin Genet* 2002;62:390-393.

Editor's comment: *The information contained in this paper should be very useful to physicians, genetic counselors and others who deal with families concerned about long term survival in Down syndrome.*

William Horton, MD

Mutagenesis Does Not Explain Paternal Age Effect in Achondroplasia

Achondroplasia is the prototype of chondrodysplasia in humans. Its major features include short limb dwarfism and a large head with mid-facial hypoplasia. Achondroplasia arises most often as a sporadic event to normal parents and there is a pronounced paternal age effect. It results from activating mutations of Fibroblast Growth Factor Receptor 3 (*FGFR3*), which encodes the transmembrane receptor. *FGFR3* mutations have several unique features including that they arise *de novo* almost exclusively during spermatogenesis and that almost all involve the same G-to-A transition at base pair 1138 (G1138A) of the gene resulting in a glycine to arginine substitution in the transmembrane domain of the receptor. Taken together, these observations have led to the commonly accepted views that *FGFR3* is exceptionally mutagenic and that the paternal age effect reflects replication errors that occur during spermatogenesis. Spermatogenesis continues throughout life and presents many more opportunities for erroneous copying of DNA than does oogenesis in which replication ceases before birth. Although this explanation makes good sense, there is now evidence that *it is incorrect*.

To test if increased mutagenesis accounted for the paternal age effect in achondroplasia, Tiemann-Boege et al determined the frequency of the common G1138A *FGFR3* mutation in sperm from 118 healthy men ranging in age from 18 to 80 years. They expected to detect a progressive increase in sperm mutation frequency comparable to the increase in number of achondroplasia births to older fathers. However, to their surprise, using a carefully controlled polymerase chain reaction assay, they found only a small increase in the G1138A mutation which by itself could not account for the paternal age effect.

More specifically, they observed that the mutation rate for G1138A averaged about 1 per 11,000 haploid genomes over all ages. Broken down by age, the mutation frequency changed little between the ages of 18 - 40 and 55 - 80 years. It increased about 2-fold between the two age groups, but this was nowhere near

the increased frequency of achondroplasia births in older fathers.

The authors addressed in considerable depth various possible explanations for their findings. Several involve experimental biases or artifacts. For example, fathers of children with sporadic achondroplasia may constitute a subgroup of men with distinct mutation properties that differ from the sperm donor population. There may be unappreciated ascertainment biases with regard to the makeup of donor population or in previous studies. Despite extensive controls, there could have been underreporting of mutations in the PCR assay. These studies may have led to overestimating the magnitude of the paternal age effect.

Two of the possibilities deserve special attention. The first is that there may be an age-dependent increase in germ-line permutations at the G1138A site that are neither converted to a full mutation or repaired before fertilization. One candidate lesion would be an unrepaired G/T mismatch resulting from deamination of 5-methyl cytosine. The cytosine at position 1138 is known to be highly methylated in sperm and therefore a candidate for such a premutation, which might go undetected under conditions of PCR.

Another possibility is that the G1138A mutation gives a selective advantage to sperm that carry it. The authors acknowledge the highly speculative nature of this possibility, but point out that FGFR3 is expressed and presumably active in mature sperm cells. They also caution that invoking this possibility must include an explanation of how a potential selective advantage would increase with age.

Tiemann-Boege et al. *PNAS 99* 2002;14952-57.

Hurst LD, Ellegren H. *Nature* 2002;420:365-66.

Editor's comment: *Many observations over the last several years have led to the dogma that FGFR3, especially the site where achondroplasia mutations arise, is extraordinarily mutable during spermatogenesis and that this mutability increases dramatically with age. The idea that DNA is prone to replication or mitotic errors, that there are many more opportunities for such errors to occur during spermatogenesis compared to oogenesis, and these can somehow accumulate with age has been conceptually appealing and is easy to explain during counseling. However, the results reported here cast serious doubt on its validity. Assuming they hold up, which seems highly likely given the considerable lengths to which the authors went to control their experiments and validate their results, the dogma will need to change.*

The notion of genetic premutation in achondroplasia is not new. It was proposed by John Opitz and others long before mutations of FGFR3 were discovered. It never gained much momentum, probably because it lacked experimental data with regard to a specific locus or mutation; however, the paper by Tiemann-Boege et al may add new life to this concept.

The possibility that sperm which harbor activating mutations of FGFR3 have a selective advantage for motility, fertilization or the like, is intriguing. Of note is that activating FGFR3 mutations found in the achondroplasia family of disorders have been detected in several types of cancer, including multiple myeloma and bladder, breast and colon carcinoma. The mechanisms through which the mutations contribute to neoplasia are not well understood. However, they may well give the cancer cells a competitive advantage over the normal cells.

William Horton, MD

Is Insulin-Like Growth Factor-1 Monitoring Useful in Assessing the Response to Growth Hormone of Growth Hormone-Deficient Children?

In order to assess the relationship between insulin-like growth factor-1 (IGF-1) and the growth hormone (GH) dose utilized to treat GH-deficient children, the IGF-1 response was compared with the changes noticed in height-standard deviation scores (H-SDS) and height velocity during treatment.

The study was carried out in 24 prepubertal GH-deficient patients with a mean age of 10.5 ± 1.8 years and a mean bone age of 8.4 ± 2.1 years. H-SDS for chronological age and bone age before therapy were -2.6 ± 0.8 and -1.2 ± 0.8, whereas height velocity was -1.1 ± 1.5 cm. Serum IGF-1 and insulin-like-growth factor binding protein-3 (IGFBP-3) levels were measured before, after 6 months and 12 months of GH treatment, and correlated with the GH dose. IGF-1 increased significantly during the first six months of therapy, but did not increase any further at twelve months, despite the use of higher GH dosages (0.14 vs. 0.1 IU/kg/day), whereas IGFBP-3 increased at both 6 and 12 months. There was no correlation between GH dose and IGF-1 and IGFBP-3 levels. Height velocity as well as height for chronological age and bone age were significantly greater after one year of treatment with GH. The authors concluded that the increment in IGF-1 during therapy did not correlate with the interval height increase and was found to be less useful than height increments in adjusting the GH dose needed to treat prepubertal GH-deficient children.

Lanes R, Jakubowicz S. *J Pediatr* 2002;141:606-610.

***Editor's Comment**: The monitoring approach that individualizes therapy and includes both biochemical and auxological determinations to titrate the GH dose utilized to treat GH deficiency is considered standard practice in treatment with GH. A common practice is to monitor height increments and serum IGF-1 and IGFBP-3 concentrations to guide with the treatment of GH-deficient patients. However, in this study IGF-1 and IGFBP-3 levels were not found useful in assessing the response to GH treatment. There are wide variations in IGF-1 levels during the day, as well as different stages throughout time, and even in the same individual. Of great importance is the nutritional status and intake of the patients in relation to the IGF levels. Any one or several of these factors might have played a role in the lack of a clinically relevant, as well as statistically significant, difference in IGF levels found in this small group of patients studied. The reader is advised to read the editorial on this paper published in the same journal by Dr. Barry Bercu[1] entitled "Titration of growth hormone dose using insulin-like growth factor-1 measurements: Is it feasible in children?" This study once again demonstrates that careful measurements of height and the monitoring of growth progression is the most important marker in the assessment of short children with or without GH deficiency, as well as during treatment with GH.*

Reference

1. Bercu B. *J Pediatr* 2002;141:601-5.

Fima Lifshitz, MD

Leptin Measurement in Urine and its Relationship to Other Growth Peptides in Serum and Urine

Leptin is a 167 amino acid product of adipocytes that has multiple physiologic effects including appetite suppression, alteration in energy balance, acceleration of pubertal onset, and both stimulatory and inhibitory effects on bone mineralization. Its role in human physiology other than for appetite suppressive effects and possible hypogonadotropism, is uncertain. The authors have adapted a two-site immunoradiometric assay (IRMA) for measurement of leptin in serum to its determination in urine. In this assay, two mL of urine (unmodified by acidification or dialysis) are incubated initially in a plastic tube coated with antibody (#1) to leptin, followed by incubation with a second, radiolabeled antibody (#2) to leptin with specificity to a different epitope. Free labeled antibody (#2) is removed and radiolabeled bound antibody (#2) quantitated. Leptin in urine (lep/u) is calculated by comparison to standards of leptin similarly prepared. Lep/u was quantitated in timed overnight urine collections in 188 (100 females) children and adolescents 5-19 years of age. Serum and/or urinary levels of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II), and IGF binding proteins (IGFBP3 and IGFBP-1) were also determined. The IRMA for lep/u was validated by dilution and recovery experiments. In the cross-sectional survey, total lep/u was similar in prepubertal boys and girls (0.2 ng/night). Lep/u values increased to a peak in boys at Tanner genital stage III (0.8 ng) and then declined; in girls, lep/u continued to increase through breast stage V (1.1 ng) and values were significantly higher in adult females than in males. The maturational patterns of lep/u were similar to those described for serum leptin (lep/s) changes. Log transformed values of lep/u and random lep/s were highly correlated. Lep/u levels were variable related to age, stage of sexual maturation, BMI, IGF-I, and IGF-II. In two adults in whom overnight urines were collected consecutively for more than 30 nights, nocturnal lep/u values varied night-to-night by 42-75%. In a substantial number of specimens (20+%) obtained from both the children and adults, lep/u was not measurable. The authors conclude that measurement of timed overnight lep/u is a feasible method for longitudinal assessment of leptin production in children, adolescents, and adults.

Zaman N, et al. *Clin Endocrinol* 2002;58:78-85.

***Editor's Comment**: The majority of secreted leptin is catabolized in the kidney to smaller peptides. The investigators relied, in part, upon the specificity of two antibodies directed to different epitopes of leptin to validate the IRMA for lep/u. However, it would have been of interest to examine the physicochemical properties of urinary leptin by size exclusion chromatography and/or mass spectroscopy to determine more accurately the nature of the peptide measured by the IRMA. It would also have been of interest to have measured urinary/serum levels of gonadotropins and sex hormones and to assess their relationships to lep/u and stages of sexual development (perhaps a manuscript already in preparation). Nevertheless, the data are of interest and the described method may be helpful in furthering our understanding of the relationship between growth, sexual maturation, and leptin.*

Allen Root, MD

Letter to the Editor: Misconceptions - Epiphyseal Fusion Causes Cessation of Growth

Dr. A. Michael Parfitt brought to the attention of the Editorial Board his article published in a journal not often reviewed by *Growth, Genetics & Hormones*. I have summarized some of the highlights of this very interesting article and recommend that the readership review the full paper, as it is of great interest.

Parfitt AM. Misconceptions: Epiphyseal Fusion Causes Cessation of Growth. *Bone* 30:2002;337-339.

This paper brings to light the fact that when the bone reaches its appointed genetically determined length, the following takes place: the longitudinal growth ceases, the epiphysis fuses with the metaphysis, and the growth plate disappears. Pediatric endocrinologists have always believed that growth stops because the epiphysis fuses, and that short adult stature could result from early fusion of the epiphyseal growth plate. The reverse is also true - a sustained linear growth through puberty could be a consequence of failure of epiphyseal fusion. However, Dr. Parfitt suggests that the epiphysis fuses because growth stops. In other words, fusion is the marker of growth cessation, not a determinant of it.

Epiphyseal fusion is an active process that might not necessarily be preceded by, nor automatically follow, the cessation of growth. Endochondral ossification represents the culmination of a sequence of changes in the cartilage cells and their associated matrix. These events must always occur in the same order, requiring a minimum period of time. It has been shown that the growth plate narrows, not because cartilage replacement occurs earlier, but because cartilage addition occurs more slowly as the rate of chondroblast proliferation declines. The growth plate does not begin to disappear until proliferation has stopped altogether. Collectively, the data demonstrate that epiphyseal fusion does not precede, but rather follows the cessation of growth. Nevertheless, fusion is not simply the result of continued cartilage replacement with no further cartilage addition; this is an active process with its own hormonal controls, cellular mechanisms and structural features. For example, if there is estrogen deficiency, the epiphyses may remain unfused long after growth has stopped, with resumption of the normal timetable of fusion after replacement of the missing hormone. However the complexity of estrogen action at the growth plate has contributed to the current confusion. Estrogen has separate and independent effects on chondroblast proliferation and on active epiphyseal fusion. It has a biphasic effect on proliferation, which is stimulated by low levels and inhibited by high levels. The latter predominate in late adolescence in both sexes, leading initially to growth cessation and subsequently to active fusion. Dr. Parfitt concludes that recognizing the correct temporal relationship between growth cessation and fusion is an essential first step to understanding the complexities of growth plate function, but evidently a great deal more work is needed to clarify all the sequences.

Editor's Comment: *The effects of the high levels of estrogens found in sexual precocity may account for the early fusion of the epiphyses and the reduced height of the patients. The biphasic effect of estrogen on chondroblast proliferation as stated by Dr. Parfitt would account for these findings.*

Fima Lifshitz, MD

Gastrointestinal Complications of Russell-Silver Syndrome

A survey was conducted among members of the support group MAGIC, which includes individuals with Russell-Silver Syndrome (RSS) and their families. Completed surveys were returned from 135 individuals. Of those, 65 were determined to have clear-cut RSS on the basis of the criteria of: small for gestational age (IUGR), small for age during childhood, and having preservation of head circumference. Asymmetry is often seen in RSS as well. To be included in the study, it was necessary for the subjects to have at least three of four findings. If they had only three distinctive minor clinical features, other features were sought, including hypospadias, clinodactyly, triangular face and hypoglycemia to confirm the affected individual as a "clear cut" case.

In carefully reviewing these "typical" RSS cases, a surprisingly high frequency of gastrointestinal (GI) symptoms were found. Among the many areas of complications surveyed, GI problems stood out. Out of 65 subjects with typical RSS, 77% (50 subjects) had gastrointestinal symptoms. The major symptoms included gastroesophageal reflux disease (34%), food aversion (32%), and esophagitis (25%). The latter two are often a result of gastroesophageal reflux.

These observations suggest that the GI problems are often significant components of typical, "clear cut" RSS. The high incidence of reflux and esophagitis resulted in Nissen fundoplications in many affected individuals (18%). The group with GI complications also showed a high frequency of hypoglycemia (36%) as

compared to the overall group (25%). Blue sclera and kidney abnormalities were also more common among those with GI complaints.

These findings have important implications for management. In IUGR children with failure to thrive and presenting with severe GI symptoms the diagnosis of RSS should be considered.

Anderson J, et al. *Am J Med Genet* 2002;113:15-19.

***First Editor's Comment**: Among children with RSS, about 10% have uniparental maternal disomy for chromosome 7. It is not yet clear whether they also have this very high frequency of GI symptoms. This type of phenotype/genotype associations needs to continue to be explored since they are so important for natural history and management.*

Judith G. Hall, OC, MD

***Second Editor's Comment**: The association of failure to thrive, gastroesophageal reflux disease, and hypoglycemia is important. Inadequate nutrient intake increases the risks of hypoglycemia. This complication must be considered and hopefully prevented in these patients.*

Fima Lifshitz, MD

Growth Hormone Deficiency in Salt-Losing Congential Adrenal Hyperplasia

This short report describes the identification of 4 children with 21-hydroxyalse deficiency with defects in the CYP21 gene who presented with growth hormone deficiency between ages 2.1 and 12.9 years of age. These children were receiving steroid replacement at traditional doses of hydrocortisone (12 – 15 mg/m^2/d) and fludrocortisone (100 – 150 mcg/m^2/d) and were compliant with their treatment. Neuroimaging in two of the children revealed small, but present pituitary glands. All four grew well with growth hormone (GH) therapy. The authors speculate that these children may have sustained pituitary damage during salt-losing crises with associated hypotension and suggest that GH deficiency be considered in children with 21-hydroxylase deficiency who are growing poorly on traditional glucocorticoid and mineralocorticoid replacement doses.

Tirendi A, et al. *Eur J Pedaitr* 2002;161:556-558.

***Editor's Comment**: Unfortunately these authors do not present the denominator. How many children, out of a population of what size with 21-hydroxylase deficiency and poor growth, is the question to be answered. How many children with adrenal crises have poor growth? Despite these obvious and important questions, the take home message remains clear. Twenty-one-hydroxylase deficiency need not occur as an isolated disorder. Children with 21-hydroxylase deficiency, as pointed out in the manuscript, are not necessarily short. It is important to carefully consider all possible causes when evaluating growth failure in any child.*

William L. Clarke, MD

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METABOLIC SCREENING IN THE NEWBORN

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INTRODUCTION

The concept of metabolic screening for the recognition, diagnosis and treatment of inborn errors of metabolism has evolved as new methodology for detection and improved treatment have become available.[1] The diagnosis of metabolic disorders is challenging because of (1)the episodic nature of metabolic illness, (2) the wide range of clinical symptoms that are also associated with more common conditions, (3) the low incidence of these disorders, (4) the consequent lack of experience among the pediatric sub-specialties, and (5) the need for specialty testing. Although the incidence of each disorder is in the range of $1:10^4$ to $1:10^7$, there are thousands of known patients with metabolic disorders. It is probable that collectively, the total incidence exceeds 1:4000. Consequently they certainly account for significant morbidity and mortality in the newborn population.

Without doubt, the most opportune time to diagnose an inborn error of metabolism is at birth. Early recognition and correct diagnosis enables appropriate treatment, without which tragic outcomes are all too common. Public awareness of metabolic diseases was all but unknown in the United States until 1964; at that time widespread neonatal testing was introduced for phenylketonuria (PKU), a disease resulting from lack of phenylalanine hydroxylase activity and affecting about 1:23,000 newborns. Since then, most states have expanded screening to a handful of additional diseases that fit the "PKU paradigm" – a treatable disease for which an inexpensive screening test is available and that has dire consequences if left untreated.[2] Currently, most states are screening for at least four disorders: PKU, congenital adrenal hyperplasia of the 21-hydroxylase type, galactosemia because of galactose-1-phosphate uridyltransferase deficiency, and congenital hypothyroidism due to defects of thyroxine synthesis.

The case of PKU screening exemplifies the benefits of early diagnosis of a metabolic disease to patients, their families and society as a whole. The benefits of finding and treating these patients far outweigh the costs of screening the entire population.

Expanded newborn screening is a very recent development that utilizes tandem mass spectrometry (MS/MS) to screen for more than 20 inborn disorders of metabolism from a single blood spot.[1-3] This review explores the development and application of MS/MS as a clinical diagnostic testing method and its impact on newborn screening.[2,4]

ACYLCARNITINES AND DISORDERS OF FATTY ACID AND AMINO ACID CATABOLISM

The driving force for applying MS/MS in clinical diagnostics was the need to analyze a class of compounds called the acylcarnitines which can accumulate from the defective catabolism of fatty acids and certain amino acids, especially leucine, isoleucine and valine.[1-3] These normal metabolic pathways are located in the mitochondria, and are mediated by coenzyme A (CoA) leading to metabolic end-products, such as acetyl-CoA. When there is a metabolic block, abnormal acyl-CoA species accumulate inside the

Highlights In This Issue

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mitochondria, and can only escape by biochemical transformation using alternate pathways. One of the most important detoxification pathways is an exchange reaction to form a corresponding acylcarnitine – a biochemical end-product that can cross mitochondrial membranes and exit the cell (Figure 1).

A patient with a defect of fatty acid oxidation typically develops symptoms after several hours of fasting, as may occur during an intercurrent illness. Reserves of glucose are exhausted and the cell switches to the fatty acid and gluconeogenic amino acid oxidative pathways as the primary energy sources. In a defect of fatty acid oxidation, abnormal metabolites can accumulate very rapidly and result in overwhelming cellular dysfunction – causing the symptoms of metabolic decompensation. Depending on the pathway affected, these symptoms can include vomiting, lethargy, respiratory distress, apnea, coma, cardiac arrhythmias, often accompanied by acidosis, ketosis, hypoglycemia and hyperammonemia. It is during such episodes that patients are at high risk for permanent neurological damage. A delay in emergency treatment of a few hours can be fatal. If intravenous glucose is administered on time, the symptoms and the biochemical abnormalities are rapidly ameliorated. The most common defect of fatty acid oxidation is medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. It may present with Reye-like symptoms, or sudden death, yet there can be affected asymptomatic siblings within the family. Severe outcomes are entirely preventable by appropriate treatment.

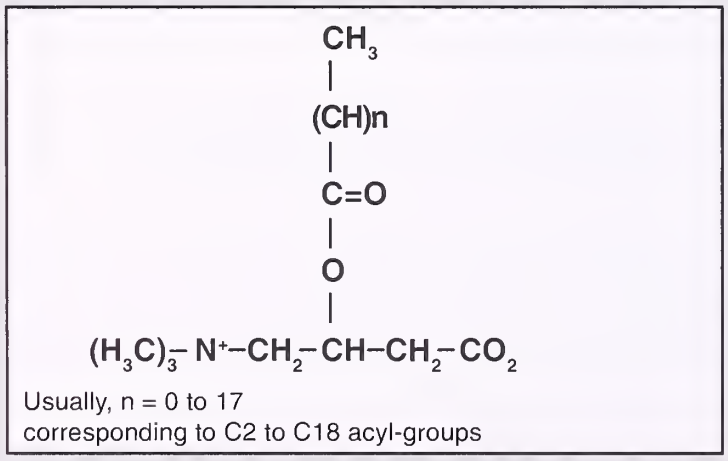
The acylcarnitines in blood reflect the primary accumulating mitochondrial acyl-CoA metabolites in disorders of fatty acid and amino acid catabolism. Thus, an acylcarnitine "profile" will recognize almost all of the defects in these pathways. While older methods cannot detect acylcarnitines, these metabolites are readily amenable to MS/MS coupled with a "soft" ionization technique such as electrospray (ESI) or fast atom bombardment (FAB).[1-3,5]

TANDEM MASS SPECTROMETRY AND THE ANALYSIS OF MIXTURES

The tandem mass spectrometer, MS/MS, usually consists of a pair of analytical quadrupole mass analyzers separated by a reaction chamber or collision cell. The triple quadrupole MS/MS is a modern system for analyzing complex mixtures. The mixture to be analyzed undergoes a "soft" ionization to create predominantly quasi-molecular ions, and is injected into the first quadrupole, which separates the molecular ions from each other. The ions then pass in order of mass/charge (m/z, ratio) into the reaction chamber or collision cell, where they are subjected to controlled fragmentation by collisions with an inert gas such as argon or helium. These fragments of the molecular ions then pass into the second analytical quadrupole where they are analyzed according to their m/z ratio. Electrospray ionisation is a 'soft ionisation' technique which enables the direct analysis of polar or high molecular weight biological substances like amino acids, acylcarnitines and proteins. These compounds can be detected and quantified directly from the solution without need to volatilize the sample. It offers excellent sensitivity (sub-picomole detection limits). Because separation of compounds in the mixture is by differences in mass spectral behavior instead of by column

Figure 1

Acylcarnitine



Usually, n = 0 to 17
corresponding to C2 to C18 acyl-groups

Structure of acylcarnitine intermediates in fatty acid oxidation inside the mitochondria. For example, in MCAD deficiency the accumulated acylcarnitine has a side chain containing 8 carbons, such that n = 7 as depicted here.

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chromatography, the entire process from sample injection and ionization to mixture analysis and data acquisition by computer takes only seconds.

The acylcarnitine "profile", generated from a small amount of blood either spotted into filter paper or after coagulation as plasma or serum, can identify more than 20 metabolic defects of fatty acid oxidation and organic acid metabolism, including MCAD deficiency (Table 1). A specimen can be sent to a diagnostic facility by overnight courier and the MS/MS analysis be completed by lunchtime on the day of arrival. MCAD gives a clear diagnostic acylcarnitine pattern as compared with normal controls (Figure 2). This is also true for most of the other disorders of fatty acid and amino acid catabolism. Thus, acylcarnitine analysis has become a valuable front-line diagnostic test for these disorders.

TANDEM MASS SPECTROMETRY AND EXPANDED NEONATAL SCREENING

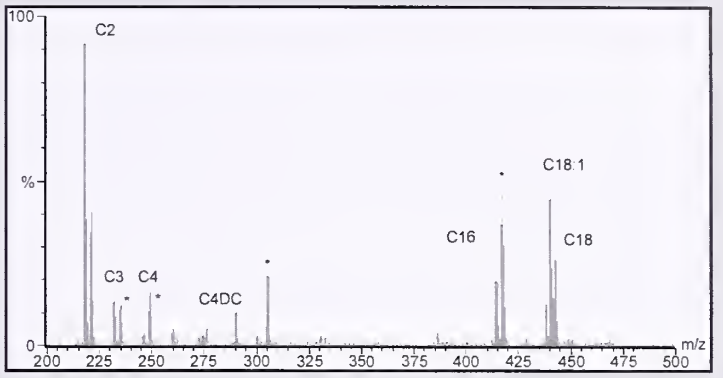
Five steps are critical to effective newborn screening: screening, follow-up, diagnosis, management, and evaluation.[4] The following sections discuss the experience with each of these steps in respect to MS/MS newborn screening.

Screening. Table 1 summarizes 2 years of initial experience by the North Carolina State Laboratory of Public Health, when 237,774 babies were screened.

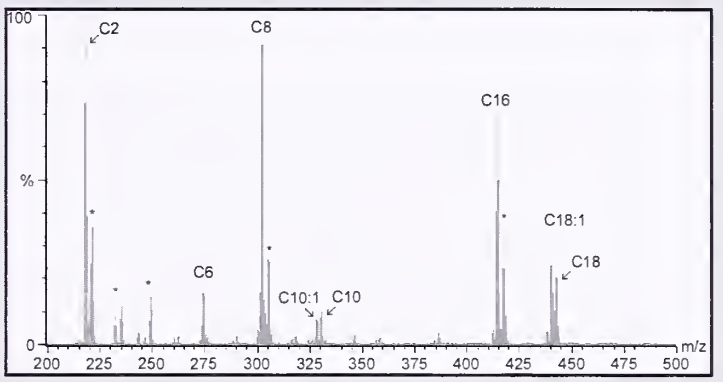
In accordance with other newborn screening programs, MCAD deficiency was detected with the highest

Figure 2

Acylcarnitine Analysis



Normal



MCAD Deficiency

Acylcarnitine profile generated by MS/MS precursor ion scan of normal plasma (upper) compared with that of a patient with MCAD deficiency (lower). Peaks represent molecular species (C2 = acetylcarnitine, etc). Note the marked accumulation of medium-chain species in the disease profile. Peaks marked "*" are added internal standards.

Table 1

Disorders of metabolism detected by MS/MS newborn screening (4/20/99 until 4/15/01)[6,7]

Fatty acid oxidation	Organic acid metabolism	Amino acid metabolism
• MCAD (medium chain acyl-CoA dehydrogenase) deficiency (21) • VLCAD(very long chain acyl-CoA dehydrogenase) deficiency (1) • SCAD (short chain acyl-CoA-dehydrogenase) deficiency (3) • GA (glutaric acidemia) type II* • CPT II (carnitine palmitoyl transferase II) Deficiency* • LCHAD/TFP (long chain 3-hydroxylacyl-CoA dehydrogenase) deficiency*	• 3-MCC (3-methyl crotonyl-CoA carboxylase) deficiency (7) • Propionic acidemia (1) • Methylmalonic acidemia (2) • Glutaric acidemia, type I (1) • β-ketothiolase (SKAT or mitochondrial acetoacetyl-CoA thiolase) deficiency (1) • Isobutyryl-CoA dehydro-genase deficiency (1) • 2-methylbutyryl-CoA dehydrogenase deficiency (1) • Isovaleryl-CoA dehydrogenase deficiency (3) • Malonic Acidemia*	• Phenylketonuria (14) • Argininosuccinic acid lyase deficiency* • Citrullinemia (1) • MSUD* (Maple Syrup Urine Disease)

*Cases of these disorders, reported by other screening programs, had not yet been detected in North Carolina. (n)= number of patients. Total number of neonates screened 237,774.

frequency. The incidence of MCAD deficiency was estimated at 1 in 13,600 live births in North Carolina. The overall incidence of disorders of metabolism detected by MS/MS newborn screening was 1 in 4,400 live births.

Beyond implications for the affected infant, newborn screening can have implications for maternal health. An association between the risk of serious complications of pregnancy, especially in the HELPP syndrome (hemolysis, elevated liver function tests and low platelets) with the occurrence of acute fatty liver of pregnancy in the mother and a fetus affected with LCHAD deficiency, was first established 10 years ago. Since then there has been a growing awareness that the presence of other fatty acid oxidation disorders, including MCAD deficiency, can also cause pregnancy complications.

Follow-up. Initial follow-up was directed according to cut-off values for each metabolite, typically set at 4 standard deviations above the mean. In the case of an abnormal value, repeat screening samples were requested. If the initial sample had a higher "alert" value, or if the second sample remained above the cutoff, the infant's local physician was contacted immediately. The possibility of a metabolic disorder was discussed and recommendations for follow-up were made. Infants were referred directly to a metabolic genetics center. If the elevated metabolite(s) did not signal a specific or life-threatening disorder, blood and urine samples were sent to the centers from the local physicians for follow-up testing.

The importance of appropriate cut-off values and adequate follow-up testing was illustrated by an infant with glutaric acidemia, type I (GA-I), initially detected on the basis of elevated glutarylcarnitine in the bloodspot.[6] Initial cut-off values for each metabolite are typically set by a statistical determination of 4 standard deviations above the normal mean, but must be adjusted up or down for some metabolites based on experience during newborn screening. Although the patient had an abnormal blood acylcarnitine profile at birth, the repeat specimen was normal; thus, newborn screening ultimately failed to indicate the diagnosis of GA-I. Newborn screening is a powerful tool to potentially diagnose presymptomatic infants; however, it should not be considered a diagnostic test. In order to allow a precise diagnosis and treatment of GA-I, we recommend a complete evaluation, including both a plasma acylcarnitine profile and a urine organic acid analysis of any patient with elevated glutarylcarnitine in a blood spot acylcarnitine profile. The North Carolina State Laboratory has adjusted the cut-off value for glutarylcarnitine to increase the sensitivity of the newborn screening test for GA-I and this is now suggested as a general recommendation for laboratories screening for GA-I by MS/MS.

Diagnosis. The diagnoses of fatty acid oxidation disorders is established by testing urine organic acids and a plasma acylcarnitine profile; whereas, the diagnoses of organic acid metabolism disorders is confirmed by plasma amino acids +/- urine organic acids. Enzyme analysis is required to diagnose disorders where the elevations of metabolites in blood and urine do not provide a conclusive diagnosis.

Since the addition of MS/MS to the North Carolina Newborn Screening Program, 20 infants with elevated hydroxyl-isovalerylcarnitine (C5OH) levels were evaluated. Eight of these 20 infants had persistent elevations of both 3-hydroxyisovaleric acid and 3-methylcrotonylglycine in their urine, highly suggestive of 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency. Other enzyme deficiencies that could provoke elevated C5OH, including biotinidase and holocarboxylase synthetase deficiency, were eliminated from the differential diagnosis by confirmatory enzyme testing. In 4 of the remaining 12 infants, maternal 3-MCC deficiency was demonstrated. It is likely that the remaining 8 of these 12 infants for whom urine organic acids normalized also represented maternal 3-MCC deficiency; however, follow-up testing was not requested from the mother or she refused to provide her samples in each case. Infants and mothers with 3-MCC deficiency commonly have clinically significant carnitine deficiency, which motivated the detection and treatment of these individuals.

Management. The prompt referral of patients with confirmed or suspected life-threatening disorders of metabolism is critical to fulfil the mission of newborn screening. The successful treatment of inborn errors of metabolism provides justification for MS/MS newborn screening. For example, untreated MCAD deficiency presents as hypoketotic hypoglycemia and is commonly lethal, due to hepatic failure which often mimics Reye syndrome. Since the initiation of MS/MS newborn screening, there have been no deaths among confirmed MCAD deficiency and no cases of missed MCAD deficiency. Treatment consisted of early referral to a metabolic-genetics center, avoidance of fasting, L-carnitine supplementation, and prohibition of formulas containing medium-chain triglyceride (MCT oil). Likewise, nutritional and pharmacologic treatment is available for other disorders detected by MS/MS.

However, the treatment of other potentially detectable disorders of metabolism has been less than optimal, related to issues of detection or delays in detection. While tyrosinemia, type 1, can be effectively treated with

a life-saving enzyme inhibitor, tyrosine levels are not elevated during the newborn period to allow detection of that disorder. More frustrating has been the ineffectiveness of treatment in disorders with severe complications early in life, including glutaric acidemia, type II (GA-II) and maple syrup urine disease (MSUD). GA-II cannot be effectively treated when the presentation is severe, and MSUD can only be effectively treated when a formula lacking branched-chain amino acids is used prior to the onset of symptoms which usually occurs in the first 10 days of life. Although treatment is available for GA-I, MSUD and tyrosinemia, type I, these disorders are quite rare outside selected population isolates (eg. MSUD among the Amish). Consequently, aggressive, earlier detection by more specialized approaches to newborn screening is not practiced.

Evaluation. Newborn screening programs require periodic review and analysis of outcome measures to be successful. Adjustment of cut-off values is one important exercise in MS/MS newborn screening, since the cut-off values determine the likelihood of false positive or false negative results.[7] False negative results should be assiduously avoided. False positive results can hamstring a program. Specific causes of false positives are listed in Table 2.

Ratios of metabolites are helpful in the interpretation of elevations unrelated to a metabolic disorder, such as the ratio of C8:C10, which is elevated in MCAD deficiency but not in MCT oil supplementation. Age-specific cut-off values could potentially reduce the frequency of false positive results because the majority of spurious elevations are related to prematurity.[7] Until age-specific cut-off values are available, the newborn screening laboratory typically obtains serial specimens from premature infants until the postconceptual age approaches 40 weeks.

The effectiveness of modifying cut-off values was illustrated by the experience with C5OH. The initial cut-off for C5OH was determined statistically (4 standard deviations above the mean); the cut-off was increased when the false positive rate was determined to be unacceptably high. Thereafter, the cut-off for C5OH was increased to 5 standard deviations. This adjustment of cut-off values for normal samples has reduced the number of initially elevated samples from 1 in 720 to 1 in 7,400 infants screened, and dramatically reduced the ratio of falsely positive initial screens to a truly positive test in affected infants from 65 to 1 to 3.3 to 1. There was no reduction in the rate of 3-MCC detection observed after the cut-off for C5OH was increased, and no infants with symptomatic 3-MCC deficiency have come to the attention of the North Carolina medical community since the MS/MS screening began.

CONCLUSION

The difference in newborn screening brought about by MS/MS is the ability to detect more than 20 inborn disorders of metabolism from a single blood dot with a single test. The method detects a confirmed disorder in about 1 in 4,000 cases screened. The most common diseases are MCAD deficiency, PKU, and 3-MCC deficiency. Early diagnosis and treatment of these cases is preventing adverse outcomes, and screening programs are reporting a very low incidence of false positives and false negatives. About half of the states are either screening newborns by MS/MS or have made a decision to do so soon. Even so, there is controversy and debate regarding what is perceived to be a paradigm shift, since the testing equipment is expensive and some of the disorders it detects have no effective treatment. However, once a state decides to implement this method it must accept the responsibility of performing the test properly and of treating diagnosed patients. To do so means providing adequate professional support to include dietitians, genetic counselors, biochemical geneticists and appropriate mechanisms in place for follow-up testing. Pediatric Endocrinologists are often called to consult with infants with emergencies due to inborn errors of metabolism, a good review of the subject should be kept at hand.[8]

Table 2

Causes for false positive results in MS/MS newborn screening

Condition	Metabolites affected	False positive
MCT oil supplementation	C8, C10	MCAD deficiency
Prematurity	C4, C5, C8	GA-II & MCAD deficiency
Prematurity	Tyrosine	Tyrosinemia
Carnitine supplementaion	C0, C2, C3, (+ others)	Propionic acidemia & others

ACKNOWLEDGMENT

We gratefully acknowledge the assistance of Dr. Diane Frazier and Dr. Joseph Muenzer of the University of North Carolina, and of Dr. Shu Chaing and Ms. Susan Weavil of the North Carolina State Laboratory of Public Health, for compilation of data and in the follow-up and evaluation of patients with abnormal MS/MS newborn screening in North Carolina.

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Letter to the Editor:

Dear Dr. Blizzard and Editors of GGH:

I've been reading GGH for years, and have found it so useful. This month's timely release of the intersex review really "hit the mark". I work in a state birth-defects surveillance department. The non-physicians have expressed tremendous interest in the management of ambiguous genitalia, either as an isolated finding, or related to exstrophy. This review will serve as the focal point for our next monthly teaching session to be supplemented by your review (*GGH* Vol. 19, No. 1).

Angela E. Lin, MD
Brigham-Women's Hospital
MA Center Birth Defects Prevention

Dear Dr. Linn: Thank you!

Dear Other Readers:
Please let us know your positive and negative comments – and your agreements or disagreements regarding the abstracts and their comments and the lead articles. Your input is absolutely necessary for us to maintain, upgrade, and disseminate your agreements and disagreements. We encourage you to respond quickly after your thoughts and criticisms come to mind.

Robert M. Blizzard, MD

Factors Determining the Pattern of the Variant Creutzfeldt-Jakob Disease (vCJD) Epidemic in Great Britain

Editorial Preface: Growth hormone (GH) extracted from human pituitaries obtained at autopsy was first given to children in 1958. Twenty-seven years later (1985), the first cases of Creutzfeldt Jakob Disease (CJD) resulting from such injections were observed in individuals who had received GH injections 8 to 10 years prior to that time. The fact that no cases of CJD were reported reflects the long latent period between exposure and the onset of symptomatic disease.

The exact number of the pituitary injections that may have been contaminated with the CJD prion is unknown. GH from only one of three laboratories in the U.S. extracting pituitaries has been associated with CJD. All three of the laboratories extracting GH used different procedural techniques. In retrospect, the GH extraction procedure of two of the three laboratories eliminated the active prion from the final product. From 1985 until April 2003 only 26 cases of CJD were recorded among several thousand (7,700) recipients in the U.S. who had received native human growth hormone. All U.S. patients with CJD received GH prior to 1977; afterward a new purification step was added to the GH extraction procedure.

The early symptoms of CJD consist of degenerative neurological function. Death unfortunately follows within a period of 6 to 36 months. The number of catastrophes to date in the United States have been relatively small, particularly in light of the number anticipated in 1985 when the first two deaths were reported within a month of each other. Postulation, with reasonable justification, was that the incubation period and susceptibility to the disease were influenced by the dose of contaminated material, possibly the age of the recipient, and possibly by an individual's genetic susceptibility. The latter was suspected on the basis of a few studies using scrapie disease in sheep as a prototype since CJD, occurring primarily in humans, is similar to scrapie disease in sheep. These diseases produce degenerative neurological alterations; although the histology of the pathological findings in the central nervous system are different. They are known as spongiform cerebral encephalopathies.

Abstract: In 1985 and 1986 a similar but different spongiform encephalopathy manifested itself in England when humans were first diagnosed with "mad cow

disease" or bovine spongiform encephalopathy (BSE). Cows had been infected by the ingestion of commercially prepared food for cows to which had been added a food enforcement consisting of bovine CNS and other organ components that were unmarketable to humans. Cows ingesting these ground up organ components, when the organs were contaminated, developed BSE after a prolonged incubation period. Infected cattle in the presymptomatic stage were often sent to the slaughter house. This meat was sold in the markets and subsequently infected humans. Thus, the mad cow disease was perpetuated and humans developed a variant of CJD (vCJD). The brain pathology of CJD and vCJD are distinguishably different even though both are spongiform encephalopathies. Over one million cows in the UK were believed to be infected. Identification of infected asymptomatic cows is not easy even though the prion accumulates in the lymphoid tissue as well as in the central nervous system.

Spongiform encephalopathies result from a replicating abnormal protein called a prion. The prions proliferate, destroy cell membranes, and accumulate as they are not destroyed themselves. Clinical symptoms develop when the abnormal protein is diffusely spread through the CNS. Transmission from mother to fetus occurs during pregnancy in the cow. It is not known whether prions are transmitted in cow's milk or colostrum. There are no data regarding transmission in humans by placenta, in human milk or colostrum.

At the end of 2001 in the UK there were 113 cases of vCJD, nine of whom were alive at that time. A few cases have occurred in other countries including France and Ireland and two cases in the United States. BSE crosses species barriers and consequently is found in squirrels and other mammals. The disease scrapie has been adapted to mice and genetic predisposition has been studied. Different strains of mice react differently to the exposure of the scrapie prion. Recently a genetic predisposition for susceptibility in humans has been demonstrated. At the time the referenced article was written, all of the human cases tested in the UK (87) shared a common genetic trait, being methionine homozygous (MM) at codon 29 of the prion protein (PrP) gene. Estimates in Caucasian populations are that 40% of the population share this trait. Of the other 60% of the population, 13% are valine homozygous (VV) and the remaining 47% heterozygous for methonine and valine (MV). The authors of the referenced article also refer to a report that there is a decreased risk of CJD in those with HLA-DQ7. This new finding, if correct, suggests complex multi gene determinations of patterns of susceptibility.

The authors discuss extensively the difficulty in predicting the potential magnitude of the UK epidemic. Of significant importance, the authors believe that even in the worst case scenario in which over 8,000 cases will appear by the year 2080, it is unlikely that a very large increase in case numbers would be expected in the short term (2-5 years).

The epidemiological determinants of the cause of the epidemic which make projections complex include; (a) incubation period distribution, (b) possible age dependent susceptibility to exposure to infection, (c) the effectiveness of the specified bovine ban in the UK, and (d) the genetic susceptibility to infection. For each of these determinants the data used for calculation are nebulous. However the best current estimate (guesstimate) of (a) for mean incubation period is stated with trepidation to be ca. 7 years, (b) the age dependent maximum susceptibility for individuals is 10-20 years of age, (c) for effectiveness of the specified bovine ban, the authors are unable to utilize current data in the calculation, and (d) in respect to utilizing genetic susceptibility, recent studies have indicated that there may be substantial genetic variation in susceptibility, which prevents more than speculation.

The authors conclude that the main priority, in view of all the above stated difficulties, is to develop a diagnostic test that is able to both detect infection early in the incubation period and which can be applied to large population samples in humans, bovine and other species.

Ghani AC, et al. *Proc R Soc Lond B Biol Sci* 2003;270:689-698.

***Editor's Comment**: Disease curses continue to befall mankind. These are often of our own making such as in the instance of man promoting "mad cow disease". Hopefully a test will be designed that permits identification early in the incubation period of the presence of the prions and thus make it possible to identify those animals affected. Much has yet to be learned about the prion and how it might be combated.*

In respect to CJD in humans who received native pituitary growth hormone from autopsied bodies, we have suffered enough, even though only 26 of over 7,000 potentially infected subjects have died. A philosophical point, which hopefully we have learned, is that treatments which physicians prescribe today may not manifest their toxic effects for many years. As the Hippocratic Oath states, and as Lawson Wilkins practiced (Growth, Genetics & Hormones Vol. 19, No. 2) and taught, "do no harm to the patient". Unfortunately we do not have a crystal ball to assist us with the decisions we must make.

Robert M. Blizzard, MD

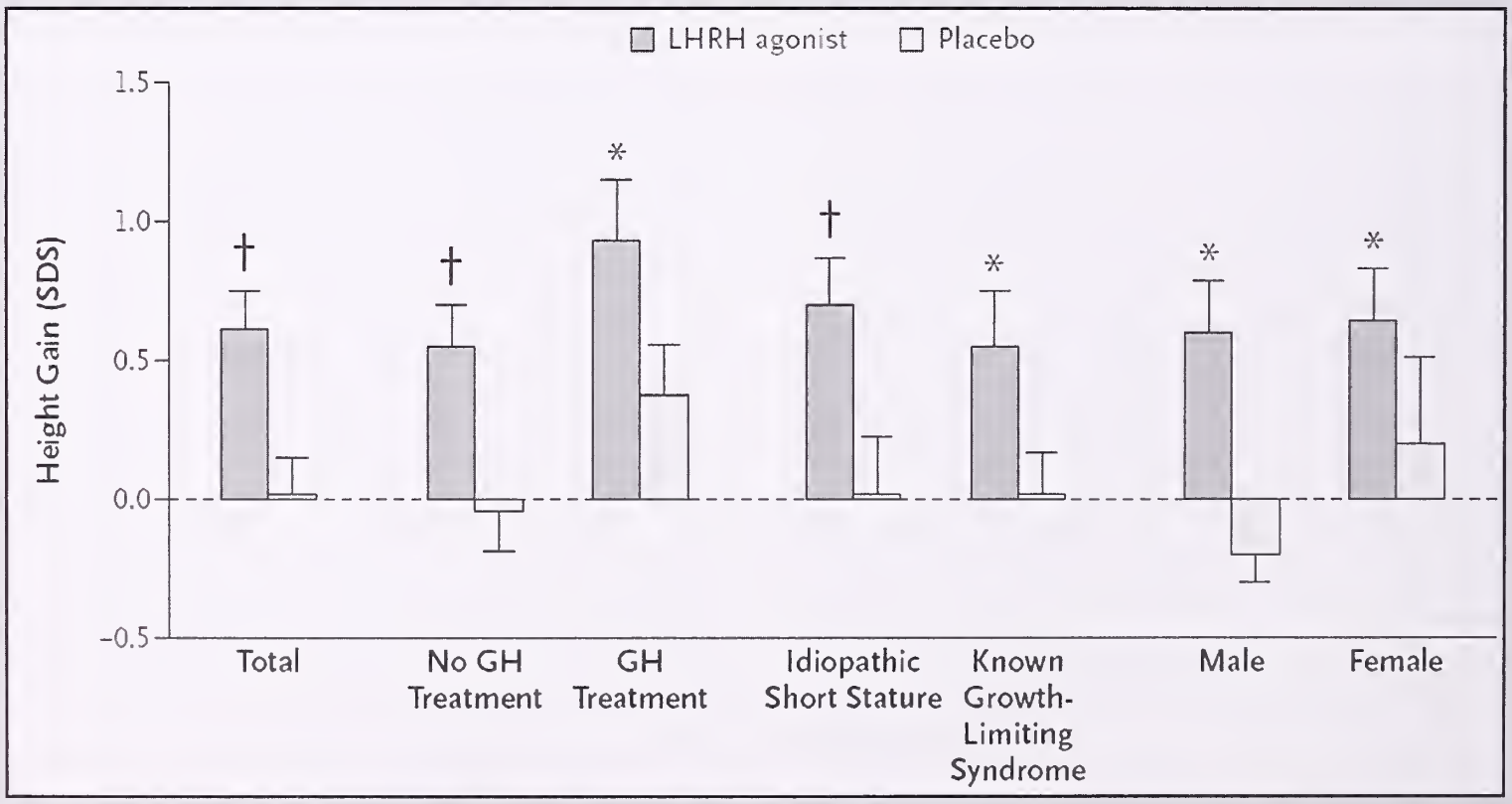
Treatment with Lutenizing Hormone-Releasing Hormone Agonist in Adolescents with Short Stature

This study was performed to evaluate whether treatment with a lutenizing hormone-releasing hormone agonist (LHRHa) increases adult height in short adolescents with normally timed puberty. There were 32 girls and 18 boys with a mean predicted adult height of more than 3 SDS below the population mean who were administered an LHRHa or a placebo in a randomized double-blind fashion; 26 subjects received the medication and 24 were given placebo. There were a variety of growth limiting disorders, but principally idiopathic short stature. Three patients were also treated with growth hormone (GH) because they had a peak GH after stimulation of less than 7µg/l. The treatment was started at approximately 12-13 years of age; mean bone age was 11.5-13.2 years, and mean Tanner stages were 2.8 to 3.2 in the two groups, respectively. The mean duration of the LHRHa treatment group was 3.5 years, and that of the placebo group was 2.1 years. Adult height was measured when the bone age exceeded 16 years in girls and 17 years in boys, and when the growth rate was less than 1.5 cm per year. Forty-seven subjects were followed until they attained full adult height.

At the end of the study, those treated with LHRHa were older and taller than those who received placebo (20 vs 18 years of age; and -2.2 vs -3.0 SD below the 50th percentile, respectively). Treatment with LHRHa resulted in a mean increase of 0.6 SDS in height (4.2 cm) over the initial predicted adult height in these short patients. The gain in height among the LHRHa treated group was independent of sex, concomitant GH treatment or presence of growth limiting syndromes (Figure). However, added GH treatment produced an apparent additive effect on growth (+ 0.4 SDS). The principal adverse event of this treatment was a decrease in bone accretion, with reduced bone mineral density below that attained in the placebo group. There were no apparent lasting effects on secondary sexual characteristics. The authors concluded that LHRHa increases adult height, but because of resulting decreased bone mineral density, it should not be routinely employed to augment adult height.

Yanovski JA, et al. *New Eng J Med* 2003; 348:908-917.

Figure



Standard-Deviation Score (SDS) for Gain in Height over the Initially Predicted Adult Height. The T bars indicate standard errors. Data are for all 47 patients with adult-height measurements. Asterisks denote P<0.05, and daggers P<0.01 for the comparison with placebo. LHRH denotes luteinizing hormone-releasing hormone, and GH growth hormone.

Reprint with permission from Yanovski JA, et al. *New Eng J Med* 2003; 348:908-917.

First Editor's Comment: *This very well controlled study clearly showed that there may be a small increment achieved in adult height (mean of 4.2 cm) with LHRHa treatment of short stature patients. Previous studies have also shown that there is a small gain in adult height with such therapy.*[1,2] *However, in this study the medication was given for more prolonged periods (mean 3.5 years) and it resulted in a significant reduction of bone mineral density. This is not surprising, since bone accretion at the time of adolescence is greatly dependent on the presence of adequate pubertal hormones which are suppressed by LHRHa. Of great concern is that this deficit persisted even after the LHRHa treatment ceased. It would have been of interest to ascertain calcium intake and determine if some of these detrimental effects could have been counteracted by an increased ingestion of this mineral. I agree with the authors that LHRHa treatment for augmentation purposes to increase height should not be routinely prescribed. The average cost of such treatment is $10,000 to $15,000 per year, and this should also be kept in mind.*

Fima Lifshitz, MD

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2. Lindner D, et al. *Eur J Pediatr* 1993;152:393-396.

Second Editor's Comment: *While the current study may not be ideal in terms of the present approach to inhibition of hypothalamic-pituitary-gonadal function with LHRHa, it is unlikely that similar investigations will be conducted in the future. Furthermore, the preponderance of girls with intrinsic short stature (32/50) without gonadal dysgenesis is the reverse of that encountered in general pediatric endocrine experience. Thus, present data serve for future recommendations. This writer agrees with the conclusion of the authors and that of the first editor's comment; namely that routine administration of LHRHa is not to be recommended for subjects with intrinsic short stature. It is of interest that the increase in adult height was greatest in patients who received both GH and LHRHa. Nevertheless, in the absence of data demonstrating significant educational, social, and occupational benefit of relatively small increases in adult stature, such efforts cannot be routinely supported.*

Allen W. Root, MD

Reference

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Do Growth Hormone (GH) Serial Sampling, Insulin-Like Growth Factor-I (IGF-I) or Auxological Measurements Have an Advantage Over GH Stimulation Testing in Predicting the Linear Growth Response to GH Therapy?

Reliable indices that are consistently able to predict the linear growth promoting effects of recombinant human growth hormone (rhGH) in short children have long been sought. The authors analyzed data from a National Cooperative Growth Study of the usefulness of IGF determinations, auxological measurements, and 12-hour serial GH measurements obtained every 20 minutes between 2000 and 0800 hours, in children who were treated with rhGH (0.29 mg/kg/week in 6± weekly injections) for a mean of 3.6-3.8 years. There were 825 prepubertal children with short stature studied (mean height -2.8 SDS; bone age delay ~2.3 years). The children were subdivided into one group of 300 (231 males, 69 females) with isolated GH deficiency (IGHD - peak GH response to provocative stimulation <10 ng/mL by unstated methods) and 525 (404 males, 121 females) with idiopathic short stature (ISS - peak stimulated GH response ≥10 ng/mL). The data were analyzed by the cluster program. In addition, a measurement of the "orderliness" or "regularity" of overnight spontaneous, endogenous GH secretion, which is termed "approximate entropy", was calculated.

As anticipated, mean and maximum spontaneous peak GH levels, pooled mean GH concentrations, and mean area under the GH peaks were significantly lower in subjects with IGHD than in those with ISS. Interestingly, pretreatment IGF-I concentrations were similar in the two groups (120 and 125 ng/mL, respectively). The increment in height SDS after treatment with rhGH was similar in the two groups (+1.2 to 1.3 SDS). Significant but weak correlations ($r < 0.4$) related rhGH-induced height increment to height deficit prior to treatment, duration of treatment, and mid parental height SDS in both groups. Maximum stimulated GH values, spontaneous overnight GH measurements, and pre-treatment IGF-I levels were also inversely related to rhGH-induced growth, but again the r values were low (-0.15 to -0.395). By multiple regression analyses, only the peak GH response to secretagogue was inversely correlated to treatment related height increment; spontaneous GH measurements were not related. When data from children with "severe" IGHD (peak stimulated GH response <5 ng/mL) or "extreme" ISS (height <-3.3 SDS)

were isolated and examined, spontaneous GH measurements were inversely related to treatment induced growth but did not improve calculated height prediction models. Spontaneous GH secretion was more orderly in children with severe IGHD than those with "moderate" IGHD. In the ISS subjects, GH secretion was more orderly in those with "mild" ISS, and IGF-I concentrations were higher than in those with extreme ISS. The authors conclude that in general, serial measurements of spontaneous overnight GH secretion did not provide information helpful in the prediction of the linear growth response to rhGH, thus supporting the conclusion of several earlier studies of this question.

Rogol AD, et al. *Clin Endocrinol* 2003;58:229-237.

***First Editor's Comment**: Clearly, the diagnosis of IGHD is fraught with difficulty as 40-80 percent of such children will have normal GH secretion as adults. Thus, there must be overlap between the diagnostic categories of IGHD and ISS in this study. In this regard it is of interest that the "disorderliness" of GH secretion was greatest in those subjects with "moderate" IGHD and both "mild" and "extreme" ISS – implying a close relationship between these groups in the regulation of GH secretion. As the investigators suggest, it may be that a defect in the "orderly" secretion of GH is translated into decreased tissue responsiveness to GH even though the absolute amount of GH secreted may be normal. Although several factors were related to height increment on rhGH, none had the high r value we seek as an "absolute" predictor of response. Hence, the search goes on!*

Allen W. Root, MD

***Second Editor's Comment**: I feel pressured to comment that a possible reason that many children appear to be GH deficient as children but not as adults is that the sex hormones stimulate GH release. We use the same threshold criteria for GH release to secretagogues in adults as children. How do we know that apparent isolated GHD children are not still partially GHD as adults? Studies are needed in this group of patients when they reach adulthood to evaluate comparison of GH response to secretagogues in adults who were not thought to be GHD as children. In such a study we might find that those diagnosed with GHD as children are still GHD as adults relative to others who were never short as children. The fact that many pediatric endocrinologists used to prime suspect GHD children with testosterone before administering secretagogues for the purpose of exaggerating the GH response in suspect GHD children supports my hypothesis.*

Robert M. Blizzard, MD

***Third Editor's Comment**: In the previous issue of Growth, Genetics & Hormones (Vol. 19, No. 2) a paper by Lanes and Jacubowitz was reviewed.[1] These authors also showed that IGF-I and IGFBP3 were not useful in assessing the response to hGH therapy. Careful measurements and monitoring of growth are the gold standards.*

Fima Lifshitz, MD

Reference

1. Lanes R, Jacubowitz S. *J Pediatr* 2002;141:606-610.

Is the Growth Hormone/IGF-I Axis Stimulatory or Inhibitory on the Aging Process?

Two recent articles published in *Science* identify the GH/IGF-I axis as playing a major role in the aging process of many species including humans. The data persuasively argue that components of this axis may negatively affect longevity. The majority of the data support the hypothesis that limited secretion of IGF-I promotes long life. A brief synopsis follows.

In yeast, down-regulation of intracellular signaling pathways that are dependent on glucose increases the life span of the organisms up to 300%. In worms (C. elegans), loss-of-function mutations of a gene called *Daf-2* encoding an ortholog of the IGF-I receptor extend survival up to 300%. In the fly, inactivation of the gene encoding the insulin receptor increases longevity up to 200%. Mice with homozygous inactivating mutations in *Prop-1*, *Pit-1*, or *Ghr* survive 25%-65% longer than do wild-type mice. Since mice with a defect in the GH receptor have high serum GH, the decrease in IGF-I signaling probably is the common factor responsible for the extended life of the mutant animals, insects, etc. Partial caloric restriction in rodents and possibly in monkeys also increases life span. Decreased synthesis of IGF-I and lowering of serum concentrations of glucose and insulin occur simultaneously with caloric restriction.

Since IGF-I acts in part by increasing transcription through the mitogen-activated protein kinase pathway (MAPK),[1] which promotes cell division and growth, attenuation of this pathway possibly reduces the potential for lethal errors in this system. Since IGF-I decreases the activities of anti-oxidant enzymes such as superoxide dismutase and catalase, there is reduced ability in the presence of IGF-I to respond to stress and

thus enhanced susceptibility to cellular damage; accordingly, inhibition of this property of IGF-I would be expected to augment the stress response. Caloric restriction also increases the longevity of the *Prop-1* deficient or Ames mouse; thus, the mechanisms by which caloric restriction and IGF-I deficiency act to increase life span may differ. In rodents, partial caloric restriction increases the immune response to infectious agents and attenuates the destructive cellular immune changes of aging. This thereby decreases the incidence of degenerative and inflammatory diseases and tumor formation.

In adult humans, hypopituitarism is associated with abnormal lipid metabolism, atherosclerosis, and early death. Yet, acromegalic subjects with excess GH secretion also have a shortened life span, and critically ill patients who receive exogenous GH have a greater mortality rate than do those with similar illnesses not so treated. Since patients with *PROP-1* deficiency or patients with inactive GH receptors (who do not have ACTH deficiency), do not succumb at an early age and may even live exceedingly long,[2] Tatar et al suggest that perhaps ACTH rather than GH deficiency is responsible for early death in humans with pan-pituitary dysfunction. The authors further suggest that pharmacological agents designed to reduce IGF-I levels be explored as extenders of life span.

insulin/GH/IGF-I and their signaling pathways may actually decrease rather than prolong life span. While experimental findings cannot always be directly translated into analysis in humans, the authors' conclusions merit consideration when we prescribe GH for our adult patients. These findings also should cause those who claim that GH is an effective anti-aging agent in non-GH deficient elderly adults to reconsider this recommendation. Body weight control combined with an efficient exercise program is likely to be far more effective in lengthening life than is administration of GH to adults without GH deficiency. The need to maintain adequate glucocorticoid replacement therapy in adults with panhypopituitarism must also be emphasized to minimize stress.

Other papers in the Science series are also worth reviewing, particularly one by Hasty et al[3] describing aging defects due to genetic abnormalities in genome maintenance (transcription, DNA repair, DNA helicase activity). Bluher et al[4] recently reported that mice with loss of the insulin receptor only in adipose tissue had extended life span. In addition, an extensive review on caloric restriction and extension of life is available on the internet.[5]

Allen W. Root, MD

References

Longo VD, Finch CE. Evolutionary medicine: From dwarf model systems to healthy centenarians? *Science* 2003;299:1342-1346.

Tatar M, et al. The endocrine regulation of aging by insulin-like signals. *Science* 2003;299:1346-1351.

***Editor's Comment**: The authors point out that glucose/*

1. Pearson G, et al. *Endocrine Rev* 2001;22:153-183.
2. Rosenfeld RG, et al.. *Endocrine Rev* 1994;15:369-390.
3. Hasty P, et al. *Science* 2003;299:1355-1359.
4. Bluher M, et al. *Science* 2003;299:572-574.
5. Masoro EJ. *Science's SAGE KE* 2003; http://sageke.sciencemag.org/cgi/content/full/sageke;2003/8/re2

Can Growth Hormone Prevent Aging?

Dr. Vance recently published an article by the above title. She concludes that antiaging therapy with human growth hormone (hGH) has not yet been proven safe or effective. Although not the first investigator to study GH in relation to body composition, Dr. Dan Rudman[1] in 1990 authored the first publication concerning the use of hGH in 12 elderly men. Dr. Vance summarizes the data in that report; the administration of hGH at approximately twice the dose of hGH used in adult growth hormone deficiency (GHD) for six months resulted in a 4.7 kg increase in lean body mass, a 3.5 kg decrease in adipose tissue, and an increase of 0.02 gm/cm in lumbar spine density, and significant increases in BPs and in fasting glucose concentrations. There were no assessments of exercise endurance, muscle strength, or quality of life. Vance points out that the follow-up to this study does not include any substantiation that hGH in elderly men does more than confirm an increase in lean body mass and a reduction of body fat (with no change generally in total body weight).

Vance appropriately criticizes the proliferation of commercial "antiaging" clinics which promote the sale of inappropriate and ineffective agents such as arginine and other agents to release growth hormone and of hGH itself. Vance chastises those who for monetary gain are so dishonest and potentially destructive of their customer's health.

The use of long-term administration of hGH in adults with no established growth hormone deficiency is appropriately deprecated as it is not known whether the effect of long-term administration of hGH in the elderly is potentially harmful. Cancer of various organs is of particular concern. The work of Chan et al is cited.[2] In 152 healthy men, the relative risk of the subsequent development of prostate cancer was increased by a

factor of 4:3 among men who had serum concentrations of IGF-I in the highest quartile as compared with those men with concentrations in the lowest quartile.

The author's complete conclusion is that there is no "current" magic bullet medication that retards or reverses aging.

Vance ML. *N Eng J Med* 2003;348:779-780.

***Editor's Comment**: This editor agrees with Dr. Vance's conclusions. I concur having initiated in 1982 the first study of the effect of hGH in elderly individuals. I and four other male subjects over the age of 55 received native hGH daily for 2.0 - 2.5 years at a dose that raised IGF-I levels from GHD concentrations to levels above the 50th percentile for young adult males. In myself there was an increase in lean body mass and decrease in free fat mass. The same occurred in two other subjects but not in subjects 4 and 5. Some element of hyperinsulinemia and glucose intolerance occurred but not overt diabetes mellitus. No overt changes in gross body configuration occurred. Subjectively there were no changes in self image, sense of well-being or libido and no changes in psychological mood. There were no changes in hair color, the rate of hair or nail growth, or disappearance of wrinkles. The study was stopped in 1985 when native hGH was no longer extracted from human pituitaries because of the development of Creutzfeldt Jakob disease in some GHD patients having received hGH. On the basis of all reports in the literature and my scientific observations among the five normal elderly patients in the study cited, I agree with Dr. Vance and most other pediatric endocrinologists, "to give hGH for purposes of attempting to alter aging in individuals who secrete GH normally for age is unacceptable unless administered under a rigidly controlled double blind study".*

Reference to the role of IGF-I in shortening or lengthening life in animals is presented in the abstract immediately preceding this one (page 42). Theoretically longevity can be shortened by the indiscriminate use of GH in mammals.

Robert M. Blizzard, MD

References

1. Rudman D et al. *N Engl J Med* 1990;323:1-6.
2. Chan, et al. *Science* 1998;279:563-566.

High Dose Growth Hormone Treatment Induces Acceleration of Skeletal Maturation and an Earlier Onset of Puberty in Children with Idiopathic Short Stature

Kamp et al report on the experience of their multicenter European randomized trial of high dose (0.07mg/kg/week) recombinant growth hormone (GH) in prepubertal idiopathic short stature (ISS) children with baseline heights less than - 2SDS. Forty children (ages 4 –10 years) were recruited and 12 completed 4 years of study while 8 completed 5 years of treatment. Inclusion criteria, in addition to pre-pubertal status and age <8 years for girls and < 10 years for boys, were normal responses to GH stimulation testing (GH >10µg/l). Subjects were measured and Tanner staging performed every three months; bone age determinations were made yearly. During the first year of treatment all subjects randomized to GH treatment participated in a "GH responsiveness" study where GH was administered at two different doses for three months each, separated by three-month washout periods. High dose GH treatment was continued until the first signs of puberty.

In the second and subsequent years of treatment, height SDS for chronological age increased significantly and there was a significant difference in bone age advancement compared to controls. Indeed, height SDS for bone age was not different between the two groups at five years. Eighty-five percent (11/13) of boys in the high dose GH group entered puberty at a median age of 12.2 years during the study, compared with 54% (7/13) of controls at a median age of 13.9 years. Similar findings for girls included 50% (2/4) of treated children entering puberty at a median age of 10.2 years versus 20% (1/5) of controls at a median age of 9.9 years. The age and sex adjusted relative risk of entering puberty earlier was 4.7.

The authors conclude that there is no evidence that young children with ISS benefit from high dose GH in the pre-pubertal period. They point out that their study differs from previous studies in that they sought to treat younger pre-pubertal children with ISS for a longer period of time with high dose GH, and that they discontinued GH at the onset of puberty so as to separate the influence of GH from that of sex steroids in pubertal growth. They are critical of other studies that did not include randomized ISS control groups, but used reference data for pubertal onset and GH dose.

Kamp GA, et al. *Arch Dis Child* 2002;87:215-220.

***Editor's Comment**: This is an interesting and well-conceived study. The use of high dose GH in ISS remains controversial, and well-controlled studies using different GH doses in different age groups are important aids in helping the endocrinologist decide whom to treat and for how long. The data from this manuscript suggest that early high dose GH treatment may improve height*

SDS for CA, but that there may be a price to pay in final height gain by entering puberty earlier. We await the data on final heights of the subjects in this study.

In an accompanying "Commentary", Clayton[1] summarizes and reiterates previous data which demonstrate that the response to GH in ISS, whether short, mid- or long-term is variable, that overall reported gains in final heights range from 3 – 9 cm in various studies, and that pre-pubertal improvements in growth velocity are dose dependent. He reemphasizes the importance of matched contemporaneous control groups and the current lack of information regarding the dose response for GH in conditions where it is currently being used.

William L. Clarke, MD

Reference

1. Clayton PE. *Arch Dis Child* 2002;87:219-220.

Low Nutrient Intake and Early Growth for Later Insulin Resistance in Adolescents Born Preterm

In this potentially very important paper the investigators study the effects of various diets in the newborn period of premature infants versus the presence at ages 15-16 years of a plasma marker for the development of insulin resistance and non-insulin dependent diabetes in adults. The marker is known as 32/33-SPI (split proinsulin). Plasma concentrations were measured in 216 mid to late adolescents (13-16 years of age) who had been delivered prematurely (mean gestational age of 31 weeks and mean birth weight of 1.4 kg). Of these preterm infants, 110 had received a low nutrient formula and 106 had received a high nutrient formula.

Not surprising, the preterm newborns fed the lower nutrient formula gained less weight prior to discharge compared to those receiving the higher nutrient formula. The specific formulas were stopped when the infants were discharged from the neonatal unit or had reached a weight of 2000 gms. At 16 years of age the children were re-evaluated and fasting serum concentrations of insulin, proinsulin, and 32/33-SPI were determined in specific assays. As adolescents, the low nutrient group had lower levels of 32/33-SPI than the levels in the high nutrient group. Levels of insulin, proinsulin and glucose were similar in the two groups.

After statistically adjusting for the effects of gender, gestational age, birth weight, neonatal morbidity, and other variables, the relationship between neonatal diet and concentrations of 32/33-SPI remained significant. Further analysis revealed that high rates of weight gain in the neonatal period (basically a surrogate for higher caloric intake) - specifically within the first two weeks after birth - were most closely related to elevated levels of 32/33-SPI in adolescence which were independent of birth weight. There was no association between values of 32/33-SPI and weight gain between two weeks of age and discharge from the nursery, discharge and 18 months, 18 months and 9 years, 9-12, and 13-16 years. Preterm adolescents, fed a low nutrient diet at birth, did not differ in stature, weight, BMI, or sum of skinfold thickness compared with premature infants who were fed the high nutrient formula or from the control group of adolescents born at term.

The investigators conclude that premature infants who were fed a low nutrient formula (albeit one that impaired neonatal weight gain) for several weeks after birth resulted in lower concentrations of 32/33-SP in adolescence, and by inference these subjects may be less likely to develop insulin resistance. They hypothesize that the risk for developing insulin resistance in low birth weight neonates is not necessarily programmed by the intrauterine environment, but also by the immediate post partum extrauterine environment as exemplified by the high nutrient formula and more rapid weight gain that accompanies this diet. They suggest that altering current feeding practices of preterm infants by lowering their caloric intake and decreasing their early rate of weight gain may prevent later development of insulin resistance, cardiovascular disease, and the dysmetabolic syndrome without adversely affecting their long-term growth.

Singhal A, et al. *Lancet* 2003;361:1089-1097.

***Editor's Comment**: Low birth weight infants are at risk for future development of the dysmetabolic syndrome (X) of dyslipidemia, insulin resistance, and type 2 diabetes mellitus.[1] It has been hypothesized that intrauterine factors that affect the fetal response to decreased blood flow or nutrient availability "program" the subsequent development of this syndrome - primarily by inhibiting tissue responsiveness to insulin. However, there is no specific explanation that explains the cellular and molecular mechanisms by which low birth weight predisposes to insulin resistance. The current work is of interest because it points to the possibility that post natal factors, in this instance rapid growth secondary to increased nutritional intake in very early life, contribute to the later development of insulin resistance. Thus, this observation affords the possibility of an intervention that may prevent this long-term complication with*

negatively impacting the overall growth of the low birth weight subject. Considered in the context of the findings is that partial nutrient restriction and growth hormone deficiency extend life in many species[2,3] including perhaps primates. Since the level of 32/33-SPI is only a marker of insulin resistance, it will be necessary for Singhal et al to continue to follow these subjects and to document the development of insulin resistance and other adverse events as, and if they occur.

Allen W. Root, MD

References

1. Goran MI, et al. *J Clin Endocrinol Metab* 2003;88:1417-1427.
2. Longo VD, Finch CE. *Science* 2003;299:1342-1346.
3. Tatar M, Bartke A, Antebi A. *Science* 2003;299:1346-1351.

Genetics, Chondrodystrophies and Other: New Potpourri

Skeletal dysplasias are distinguished by what part of the skeleton and/or bone is involved in various types of short stature. Metaphyseal dysplasia (MCD) refers to a group of skeletal disorders in which the diagnostic findings primarily involve the metaphyses of the tubular bones. Other bones are usually normal or only slightly affected. The metaphyseal involvement may be mild (as in Schmid's MCD) or more severe (as in Jansen's MCD). Some MCD syndromes have associated extra-skeletal features (e.g. MCD – McKusick type which is also known as Cartilage Hair Hypoplasia). There appears to be a new type of chondrodysplasia with a distinctive pattern of involvement, as described by Lee et al. An eight-year-old boy with a distinctive form of metaphyseal chondrodysplasia and a previously described family with 4 generations affected are the focus of Lee's report. The child had short stature and the birth weight was 3 kg. Bilateral genu varus and wrist swelling were first noted at 4 years of age. The mother had mild wrist flaring. She was not disproportionate by U/L ratio. At 8 7/12 years the boy's height was -2.9 SD below the mean and his U/L ratio was 1.21 (normal 1.0). No significant differences were noted in the length of the upper versus the lower part of each extremity, the spine, the facial configuration or the hair. Skeletal survey revealed metaphyseal abnormalities affecting proximally and distally the tibias, fibulas, femurs, humeri, radii and ulnar bones and the hands, but the spine was unaffected. The physical and radiological findings did not fit the Schmid, McKusick, or Jansen types of MCD. A very rare autosomal dominant 4 generation affected condition described by Rosenberg and Lohr (E*ur J Pediatr* 1986;145:40-45) has features similar to those of this patient, except the patients in this family reportedly had a wedge deformity and platyspondyly of the spine which Lee et al believed to be within the range of normal variance. No molecular studies were reported in the four patients reported by Rosenberg et al or in this 8-year-old boy.

Lee YS, et al. A distinctive type of metaphyseal chondrodysplasia with characteristic thickening of the distal ulna and radius: Possible MCD–Rosenberg. *Am J Med Genet* 2003;119A:50-56.

Another example of describing a chondrodystrophy by the sites where skeletal abnormalities occur is rhizomelic chondrodysplasia punctata (RCP). This rare autosomal recessive disorder has severe shortening of the proximal long bones (rhizomelia), bilateral cataracts and severe growth and psychosocial delay. White et al report the natural history of rhizomelic chondrodysplasia punctata. Radiographic evidence of stippled epiphyses is present and MRI examination of the cervical spine is often abnormal (kinking without compression of the cord and/or compression of the cord). All children with RCP are born with severe joint contractures that improve with time although not before many of the patients (40-85%) die by one year of age. Less than 10% of the 48 cases described in respect to death were alive by 12 years of age.

Biochemical analysis and complementation studies allowed separation of the 97 patients whose data were tabulated to be differentiated on the basis of peroxisomal enzymes into three types: (Type 1) a spectrum of PEX7 gene mutations, (Type 2) mutations in the acyl-CoA:diOHacetonePO4 acyltransferase (DHAPAT) gene, and (Type 3) mutations in the ADAPS (alkyl-diOHacetonePO4 synthesis) gene.

The value of this article by White et al is that there has been a sincere attempt to delineate the natural history of RCP. The authors systematically address health concerns that arise in infants and children with RCP. The intent of White et al is to present evidence-based guidance to care providers so they can better help families understand and cope with this diagnosis. For example, 90% of infants survive for the first year and 50% survive until 6 years. Previously, death was believed to almost always occur early in infancy or childhood. Medical personnel or parents concerned and/or involved with patients with suspect or proven diagnosis of RCP are strongly encouraged to read the complete article.

White A, et al. Natural history of rhizomelic chondrodysplasia punctata. *Am J Med Genet* 2003;118A:332-342.

Other examples of chondrodystrophies are those in the subgroup known as spondylo-epi-metaphyseal dysplasia (SEMDs) which includes a number of disorders each defined by the combination of vertebral, epiphyseal, and metaphyseal anomalies present.

One such entity is the Dyggve-Melchior-Clausen Syndrome (DMCS) which is characterized by short trunk dwarfism (<-4SD) with specific radiological appearances most likely reflecting abnormalities of the growth plates including platyspondyly (flattened peripheral bodies) with notched end plates, metaphyseal irregularities, laterally displaced capital femoral epiphyses, and small iliac wings with lacy iliac crests. Mental retardation is an inherent part of the syndrome. DMCS is progressive and clinical features are reminiscent of a storage disorder, specifically Morquio's disease, but the two conditions can be differentiated by the absence of corneal clouding, deafness, valvular disease and/or mucopolysaccharidurias, all of which are characteristic of Morquio's disease.

Ghouzzi et al have used a positional cloning strategy to identify the DMC gene. They detected 7 deleterious mutations within a gene predicted from a human transcript (FLJ20071) in 10 DMC families. The DMC gene transcript is widely distributed but appears abundant in chondrocytes and fetal brain. The authors cannot explain the function of the gene product at this time, but conclude that the DMC syndrome results from loss of function of a gene that they propose to name Dymeclin, which may have a role in the process of intracellular digestion of protein.

Ghouzzi VE, et al. Mutations in a novel gene dymeclin (FLJ20071) are responsible for Dyggve-Melchoir-Clausen syndrome. *Hum Mol Genet* 2003;12:357-364.

A fourth example of types of chondrodysplasia and how they are designated is the entity called acrocapitofemoral dysplasia which is characterized by short stature of variable degrees with short limbs and brachydactyly. It is included in the differential diagnosis of hypochondroplasia. These patients also have large heads and often have pectus deformities. Epiphyseal changes are present at the shoulders, knees, ankles, hands, hips and proximal femurs. The latter are egg shaped with very short femoral necks. Shortened tubular bones characterize the brachydactyly. Congenital anomalies are limited to the skeletal system and intelligence is characteristically unaffected.

Homozygosity mapping by descent was performed in two consanguineous families. The Indian hedgehog gene (IGG) was found to be mutated in affected individuals. The nucleotide changes are seen in the amino terminal signaling domain, which is responsible for short and long range signaling. Thus, it appears to affect the regulation and proliferation of the hypertrophic chondrocytes in the growth plate. The authors postulate that the mutations cause an increased rate of chondrocyte differentiation by diminished Indian Hedgehog signaling in the growth plate.

Hellemans J, et al. Homozygous mutations in *IHH* cause acrocapitofemoral dysplasia, an autosomoal recessive disorder with cone-shaed epiphyses in hands and hips. *Am J Med Genet* 2003;72:1040-1046.

***First Editor's Comment**: The genome project has made identification of mutated genes relatively easy to identify. The effects of different mutations of the same gene has been particularly evident among the chondrodystrophies, both in relating two different entities to different mutations of the same gene and differentiating and identifying different gene abnormalities for what used to be thought the same disease entity. Unfortunately descriptive names are often misleading because there is tremendous overlap among these entities. The most recently updated classification of skeletal dysplasias can be found at www.csmc.edu/genetics/skeldys.*

Judith Hall, OC, MD

***Second Editor's Comments:** One is struck by the clinical resemblance of acrocapitofemoral dysplasia (ACFD) to achondroplasia. The phenotype is not identical, but the rhizomelic shortening of limbs, large head with prominent forehead, narrow thorax, bowing of the knees and even overgrowth of the proximal fibula on X-ray are similar. The reason for this resemblance may lie in the relationship of Ihh to FGFR3, which is mutated in achondroplasia, in the growth plate. Both regulate chondrocyte proliferation: Ihh positively and FGFR3 negatively. In ACFD the positive effect on proliferation is lost; however in achondroplasia the mutations are activating in nature so that they enhance the anti-mitotic effects of FGFR3. In other, both lead to reduced chondrocyte proliferation. A consequence of the anti-mitotic effects of FGFR3 mutations in achondroplasia is a reduction in the number of terminally differentiating chondrocytes. Since these cells are the source of Ihh, the achondroplasia mutations secondarily reduce the production and local effects of Ihh. Thus, these two disorders look alike to clinicians because they involve disturbances of the same regulatory pathways in the growth plate.*

William Horton, MD

Size at Birth and Early Childhood Growth in Relation to Maternal Smoking, Parity & Infant Breast-Feeding: Longitudinal Birth Cohort Study and Analysis

The relationship between maternal smoking, parity and early breast or bottle feeding to size at birth and childhood growth were evaluated. A large representative birth cohort was studied between 0 and 5 years of age. A total of 1335 normal infants had weight, length, height and head circumference measured at birth and subsequently up to ten occasions until they were 5 years of age. Multilevel modeling was used to analyze the longitudinal growth data. Infants of maternal smokers were systematically small at birth when compared with infants of non-smokers. However, these infants showed complete catch-up growth over the first 12 months of life. Infants of primiparous pregnancies were thin at birth and showed dramatic catch-up growth, and were heavier and taller than infants of nonprimiparous pregnancies from 12 months onwards. Breast-fed infants were similar in size at birth to bottle-fed infants, but grew more slowly during infancy; differences in weight and length persisted throughout the study period. Among infants who showed catch-up growth, males caught up more rapidly than females. The authors concluded that early postnatal growth rates are strongly influenced by a drive to compensate for antenatal restraint or enhancement of fetal growth by maternal uterine-factors.

Ong KKL, et al. *Pediatr Res* 2002;52:863-867.

***Editor's Comments**: This very interesting paper provides unique longitudinal growth data from a large prospective birth cohort. Some of the factors studied are well known to alter growth, such as maternal smoking which inhibits growth in utero, and/or breast milk which is known to be to be associated with lower growth rates in infancy as compared with cow-milk formula fed children. However, little data existed for long-term measurements of these types of infants up to 5 years of age. This paper contributes significantly with strong data. Although it is reassuring to note that infants born to mothers who smoke during pregnancy exhibit catch-up growth with no long-term consequences in height, the negative effects of smoking should not be overlooked as they transcend growth. These were not studied in this paper.*

Of great interest is the long-term growth divergence in breast-fed infants as compared to bottle-fed infants. This difference in growth progression persists after infancy with significant differences throughout the first 5 years of life. Both weight and height were decreased in the breast-fed group as compared to the bottle-fed group. It is now known that the way infants grow in utero, as well as during the first year of life, might have very important consequences for the development of adult-onset disease. Similarly, the rate of weight accretion during infancy and childhood might play a role in the development of obesity later in life. These data provide evidence that human milk feedings are best for feeding infants, allowing them rates of weight gain for the first 5 years of life that may be more compatible with a more appropriate body weight later in life. In light of the current epidemic of obesity, any factor that may contribute to it should be seriously considered. The growth charts for breast-fed infants developed by the CDC (http://www.cdc.gov/growthcharts/) and by the Eurogrowth study (www.Eurogrowth.org) are very useful in monitoring the growth of such children, but these do not extend until 5 years of age; such would be highly desirable in light of these data.

Fima Lifshitz, MD

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DR. ROBERT M. BLIZZARD - A LEGACY

Fima Lifshitz, MD
For the Editorial Board

Growth, Genetics and Hormones (*GGH*) has been published without interruption for the past 19 years. This journal was conceived and founded in 1984 by Dr. Robert M. Blizzard; the first issue appeared in March 1985. The goal set by him and the editorial board was to integrate reports of current advances in the fields of growth, genetics, endocrinology, metabolism and nutrition by bringing the most pertinent papers, with erudite editorial comments, to the attention of pediatricians, internists, pediatric endocrinologists, geneticists, nutritionists, nurses, and to others interested in these fields.

As Editor-in-Chief, Dr. Blizzard has worked tirelessly since the inception of the journal. He has been personally responsible for selecting, recruiting and stimulating the editorial board. He has elicited the best from all of us. Initially the editorial board consisted of Drs. David L. Rimoin, Fima Lifshitz and Alan Rogol from the United States, Judith G. Hall from Canada, and Dr. Jürgen R. Bierich as a European representative. Subsequently other distinguished Pediatric Endocrinologists from Europe joined the editorial board, including Drs. Jean-Claude Job and James Tanner. The current editorial board members, serving *GGH* since 1993, are Drs. William Clarke, William Horton, and Allen Root, plus founding members Judith G. Hall and Fima Lifshitz. Dr. Blizzard has spearheaded all aspects of the publication including the content, quality, and format.

Throughout the last 19 years *GGH* has exceeded his goals and has become a well established resource for all 6,000 of its current readers, many of whom cherish the journal and keep each issue in their reference libraries. As well, Dr. Blizzard has made sure that as the cycle of life continues there would be a positive and productive transition for *GGH*. During the past two years he has fostered a smooth passage to ensure that upon completion of his tenure as Editor-in-Chief the journal will continue to serve the needs of our colleagues and continue to grow. He personally has overseen all transitional aspects and bestowed responsibility for the future of *GGH* to me as Editor-in-Chief.

Dr. Blizzard requested that a short announcement be inserted about his retirement in this his last issue Vol. 19 No. 4. He wished to see that the many readers who have read *GGH* throughout the years were thanked and appreciation was expressed to all those who have contributed to *GGH* by writing lead articles and to those who have been consistent readers. We pass this message along for him, and the editorial board joins him in saying "thank you".

The editorial board, wishing to acknowledge the many years of service and the most important contributions of Dr. Blizzard, has prepared a brief outline of the accomplishments of this founding editor, teacher, pediatric endocrinologist, clinician, scientist, and man described below. This tribute to him is but a token way to bid him farewell and to imprint his legacy, so that future generations of our colleagues also may be inspired by him.

First and foremost, Dr. Blizzard will be remembered and recognized as a teacher and educator. He is an accomplished teacher, and his competence as an educator and preceptor is well known. He was trained (1955-1957) by Lawson Wilkins and he was "trained to

Highlights In This Issue

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train", when there were only approximately 20 pediatric endocrinologists in the country. He prides himself in being a pediatric endocrinologist for 48 years (1955-2003) and throughout his career he set the course for his students. Over 50 fellows, including myself and other members of the editorial board, undertook and completed their training with him. Forty-five of these are now in academic positions. Many are full professors including three deans, an associate vice president for health affairs, several chiefs of staff of children's hospitals, and several pediatric department chairpersons in the U.S. and abroad. He is proud of the fact that most of his fellows have established their own pediatric endocrinology training programs, and thus provided an ongoing transmission of the teaching of Lawson Wilkins and himself to second and third generations of pediatric endocrine fellows. He has received multiple teaching awards including those from the Johns Hopkins Hospital, the University of Virginia, and other prestigious universities. Very possibly the teaching award of which he is most proud is his election to alpha omega alpha in 1970 by the members of the Johns Hopkins Alpha Omega Alpha (AOA) Society. His accomplishments as a student had not qualified him for AOA membership, and, therefore, his election by the student membership was particularly gratifying, since only one faculty member per year was elected to the society.

He also is proud of the opportunity to have served as Acting Chairman of the Department of Pediatrics at the Johns Hopkins University School of Medicine (1972 and 1973) and as Chairman of the Department of Pediatrics at the University of Virginia, School of Medicine (1974-1987). At these institutions he fostered 15 generations of pediatric residents who sought and attained their pediatric training in his departments. Most of them are now in academic and/or clinical practice in the US. A significant number are abroad. For his educational activities he has been honored with other prestigious awards. Among them are the Ayerst (1973) and Williams (1994) distinguished service and leadership awards bestowed by the American Endocrine Society. Recently he has been honored to be elected to the Johns Hopkins Society of Scholars (2002) and honored by the establishment (2002) of the Robert M. Blizzard Annual Lectureship at the annual meetings of the Lawson Wilkins Pediatric Endocrine Society (2002). He also has been honored by invitations to deliver over 150 named lectureships and visiting professorships at many national and international academic institutions. He was elected to the prestigious Hall of Fame of Miami Children's Hospital in 1997. Those who attended the teachings of Dr. Blizzard have always recognized his talents in teaching, and most have asked for more!

However, his contributions as an educator transcend the traditional teaching role through which he personally touched so many individuals and imparted his knowledge. Dr. Blizzard made major contributions to continuing medical education by serving on multiple editorial boards of journals, editing and publication of several textbooks, and by his 19 years of editorship of *GGH*. The number of physicians and other scientists whom he reached via this journal through the years cannot be easily counted nor measured, but *GGH* is currently read regularly, as previously stated, by over 6,000 colleagues world-wide. Thus, the impact of Dr. Blizzard as an educator can be summarized as "the teacher par excellance".

In the field of endocrinology, he is particularly known for his contributions in the areas of growth and in autoimmunity, with over 200 original peer-review papers published in the literature. His picture is a clear testimony to his legacy as a clinician. He has always, in the Wilkins' style, promoted accurate measurements of children in assessing growth. This is still the gold standard in the evaluation of children with short stature. Dr. Blizzard was a pioneer in this field, publishing his first studies on the action of human growth hormone in 1959, one year after the first publication by Dr. Raben of the use of human growth hormone in growth hormone deficient individuals. His interest continues in this field to this day. Dr. Blizzard, along with Dr. Joanne Brasel and Dr. James Wright in the early 1960s published several important papers that changed the approach to the diagnosis and treatment of growth hormone deficiency. Included were the observations that growth hormone deficiency can be manifested by delayed growth even in the first year of life, previously not thought to be the case, and that the

Robert M. Blizzard, MD

acute metabolic response to human growth hormone did not correlate with its growth promoting effects when growth hormone deficient children were treated. The search for reliable indicators to predict a quantitative response to growth hormone is still ongoing.

Subsequently, Dr. Blizzard authored or co-authored 56 publications in peer-review journals pertaining to growth hormone or growth factors. These studies clarified the role that growth hormone played in producing the adolescent growth spurt, and the phenomenon of growth hormone production and its relationship to steroid production during this stage of life. A series of articles published under his tutorage unequivocally demonstrated that growth hormone increases at the time of adolescence when testosterone is produced in males. These studies showed that growth hormone and testosterone each have separate mechanisms of action in promoting growth, as well as permissive actions in the relationship to the secretion of each other.

In 1971 Dr. Blizzard stimulated his associates to design a pump that would permit a constant withdrawal of blood over a 24-hour period, that would permit the measurement of integrated concentrations of circulating hormones. Dr. Avinoam Kowarski was successful in this endeavor, and he and Dr. Robert Thompson, Dr. Claude Migeon, and Dr. Blizzard first reported the determination of integrated concentrations of human growth hormone and true secretion rates of human growth hormone. The importance of pulsatility and the intricacies of growth hormone production at various stages of life were subsequently delineated using this technique in studies with Dr. Alan Rogol, Dr. Paul Martha, Dr. Nelly Mauras, Dr. Kathleen Link, and others at the University of Virginia.

While being a leader throughout his life and an innovative initiator of investigative protocols, he appropriately was appointed Director of the Clinical Research Center at the University of Virginia (1980-1983), while serving simultaneously as Department Chairman. He collaborated extensively with his colleagues in the Divisions of Endocrinology in Internal Medicine (Dr. Michael Thorner in particular among others). He coauthored 15 papers concerning the effect of growth hormone releasing hormone in humans - both as a diagnostic and therapeutic agent.

Although not as well known, Dr. Blizzard initiated and significantly contributed in elucidating the possible role of decreased growth hormone production during adult life in the aging process. He, his associate Dr. Ann Johanson, and his group initially demonstrated that older males secrete less growth hormone than do young males, and that older males receiving growth hormone retain nitrogen, comparable to that seen in growth hormone deficient young adults. They also reported that growth hormone administered to older males generated insulin-like growth factor I, comparably to that generated in growth hormone deficient children. He subsequently described the changes in pulsatility of growth hormone secretion in older men and women as compared to younger subjects.

These studies led to the involvement of Dr. Blizzard in the first study to evaluate the effect of chronic growth hormone administration in older males. His research was not only at the intellectual/research level; he was the first of five males in a study which he initiated to receive growth hormone every day over a period of 30 months. He and his colleagues demonstrated that growth hormone had no significant effect upon skin collagen and its amino acid composition. He was obliged to stop the study in 1985 because of the report of possible contamination of native pituitary extracts by the prion producing Creutzfeldt-Jakob disease. However, the results of this project undoubtedly stimulated other investigators to assess the effect of growth hormone in the elderly.

Another major contribution of Dr. Blizzard was the concept that psychosocial dwarfism (also called emotional deprivation, maternal deprivation, the garbage can syndrome, and reversible hyposomatotropism) resulted from transient growth hormone deficiency. He insists that major credit in the concept be accepted by Dr. Dagfinn Aarskog, Dr. Gerald Powell, Dr. Salvatore Raiti, and others. The demonstration of the pathophysiology of such alterations gave great impetus to studying how the hypothalamus and its neurotransmitters are controlled by higher cerebrocortical centers which has been the subject of countless studies. To date Dr. Blizzard continues to be considered a world authority on psychosocial dwarfism or reversible hyposomatotropism.

Although currently known by young pediatric endocrinologists and academicians more for his work in the field of growth, he contributed significantly in other fields of endocrinology. In 1959 he initiated a study to determine aldosterone excretion in virilizing adrenal hyperplasia. He was the lead author of an article in the *Journal of Clinical Investigation* demonstrating that salt-losing congenital virilizing adrenal hyperplasia was due to decreased aldosterone secretion.

Between 1955 and 1980 Dr. Blizzard was a leading international investigator and authority on autoimmune endocrine diseases. He suggested that many endocrine diseases characterized by glandular atrophy, including adrenal insufficiency, hypoparathyroidism, premature ovarian failure, and insulin dependent diabetes mellitus were of autoimmune origin. He studied this model in his laboratory over the next 25 years and applied the

findings in the clinic setting which led to publications of 27 papers in peer-review journals.

In his laboratory with the assistance of Dr. Robert Chandler, he was one of the first investigators to demonstrate that Addison's disease was frequently of autoimmune origin and the first to elucidate the physical and biochemical characteristics of the antigens involved. In 1966 he reported that hypoparathyroidism was also related to antibody formation. In 1960 he had demonstrated that there was a high incidence of antibodies against thyroid microsomes and thyroglobulin in the serum of mothers of athyreotic cretins. Dr. Blizzard postulated that autoimmune thyroid disease in the pregnant woman might be the etiology of at least some cases of congenital athyreotic cretinism. At the Pediatric Endocrine Research Meetings in May 1987, Dr. Dussault of Canada presented confirmatory evidence of this hypothesis, and acknowledged that the concept and early data had been presented by Dr. Blizzard years previously.

In the early 1960's he proposed that some cases of insulin dependent diabetes mellitus were probably of autoimmune origin with destruction of the beta cells of the pancreas. This observation was based on his earlier papers reporting the associations of diabetes mellitus with Addison's disease and hypoparathyroidism. In 1961, he submitted a grant to the National Institutes of Health (NIH) proposing to study this concept. The grant was rejected stating that the concept was preposterous; subsequently it was demonstrated that indeed many patients with insulin dependent diabetes mellitus had antibodies to beta cells and the role of autoimmunity in insulin dependent diabetes mellitus was firmly established.

Even subsequent to 1979, when Dr. Blizzard was devoting the majority of his investigative time to problems of growth, he published major papers concerning autoimmunity. These papers further elucidated the associations of various types of autoimmune diseases, and particularly clarified the associations of the various types of polyglandular autoimmune adrenal disease with other endocrine disorders. At an international autoimmune conference held in Pisa, Italy, in 1979, Blizzard proposed a classification of polyglandular autoimmune diseases, which was accepted internationally and continues to be used today with only minor modifications.

Blizzard has recorded many other "firsts" in the field of pediatric endocrinology, including, with the collaboration of Dr. Ann Johanson and Dr. Harvey Guyda and other fellows, the elucidation of the intricacies of luteinizing hormone and follicle stimulating hormone secretion during childhood and puberty, and the abnormalities found in sexual precocity. In his laboratory, along with Dr. Robert Penny, he demonstrated that gonadotropin levels were elevated in hypothyroid children who have associated sexual precocity. He reported with Dr. Johanson that patients with gonadal agenesis or Turner syndrome grew significantly when treated with anabolic agents.

Other firsts included a description and report of the Johanson-Blizzard syndrome of congenital anomalies in congenital hypothyroidism and a description of the syndrome of congenital adrenal cortical-unresponsiveness to ACTH with Dr. Claude Migeon. In addition, Dr. Blizzard actively contributed to and participated in the treatment and research of patients with central sexual precocity utilizing gonadotropin releasing hormone agonists (GHRHa) to block pubertal development. Dr. Blizzard was proud of his capability to work collegially and collaboratively with others to promote multicenter investigation. An example was his collaboration over several years with Dr. Paul Boepple, Dr. William Crowley, and others in Boston in studying the role of GnRH analogues.

In 1961, in association with Dr. Alfred Wilhelmi, Chairman of the Department of Biochemistry at Emory University, the National Pituitary Agency was established. The purpose of this agency was to collect human pituitaries at autopsy examination, extracting their hormones, and to distribute these hormones on a national basis for investigation and therapy. He organized this collection and distribution program under the auspices of the (NIH), and was the Director of the agency until 1967. Dr. Blizzard inspired and led a lay group of individuals to develop an organization of parents and others to assist in the collection of human

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pituitary glands. Their success led to the establishment of the Human Growth Foundation in 1965. The scope of this organization grew and eventually became a support source for families of children with growth disorders with chapters across the country and with an ability to fund research in the area of growth disorders. After the National Pituitary Agency and The Human Growth Foundation were firmly established, Dr. Blizzard was followed by Dr. Salvatore Raiti, one of his former fellows, as director. It was this program that led to, and made possible, all of the investigation pertaining to pituitary hormones that occurred in humans in the subsequent 24 years (1961-1985) before synthetic growth hormone became available.

In 1993 he was asked to establish the Genentech Foundation for Growth and Development, a grant awarding organization separate from Genentech Inc., with an independent board and decision making authority. In the 8 ½ years of its existence under his leadership this foundation provided more than $18 million dollars in grants to clinical investigators, to basic science investigators, to physicians receiving training in the fields of growth and development, and to support professional and personal education of growth and development in these fields.

Discussing the many contributions of Dr. Blizzard to pediatrics and to science is an easy and enjoyable endeavor, particularly because he always attempted to recognize the contributions of those with whom he worked professionally. Examples of his appreciation for professional collegiality and recognition are cited in the text above. A major professional colleague of Dr. Blizzard and contributor to the success of Growth, Genetics & Hormones for 19 years is Ms. Juanita Bishop, his trusted and dependable assistant of over 20 years.

Describing the human qualities of Dr. Blizzard also is an easy and enjoyable endeavor. He is an exceptional human being, and it is worth noting the comforting way he talked to his patients and families and his ability to put them at ease despite their difficult problems. He has a special skill to develop closeness with others lasting a lifetime, and to nourish and support his patients, students, fellows, and associates. This is what I and his other associates appreciate the most!

The cycle of life continues, with the publication of this issue Dr. Robert M. Blizzard has officially retired from the editorship of *GGH*. He has had a most prestigious and distinguished career with enough accomplishments for many lifetimes. He now plans to enjoy more time with his family. We anticipate he will continue that which he does best, inspiring and teaching. As he has thanked so many of us, we thank him for all!

Screening Newborns for Inborn Errors of Metabolism by Tandem Mass Spectrometry

Newborn screening for inborn errors of metabolism has been in place in many countries for many years. Strong arguments have been made for screening not only for improving care of patients identified through screening, but also for reducing the cost of this care. Indeed, there are numerous examples, PKU most notably, of how early diagnosis and treatment have prevented serious illness or death from these disorders. However, as Wilcken and colleagues point out, formal evidence for the clinical effectiveness of screening is lacking, especially for rarer diseases, such as inborn errors of metabolism. Randomized, controlled trials of screening have been very limited because of the rarity of these disorders and also because of the strong conviction based on clinical experience that there is a benefit from early diagnosis.

Against this backdrop Wilcken et al compared the effectiveness screening for inborn errors of metabolism in all newborns with tandem mass spectrometry from 1998 to 2002 to conventional biochemical screening performed because of clinical suspicion from 1974 to 1998. The study population lived in New South Wales (Australia) and the Australian Capital Territory and totaled six million. Thirty-one disorders were selected for study. PKU and pterin disorders were excluded because effective screening by other methods had been in place for many years; also excluded were disorders known to be benign or of maternal origin.

The diagnosis rates were reported in four-year brackets, i.e., 1974-1978, 1978-1982 ... 1998-2002, etc. During the six four-year periods preceding the implementation of tandem mass spectrometry screening, 22-34 cases were diagnosed per period giving rates from 6.6 to 9.0 cases per 100,000 births. Diagnoses were made at different ages depending on the age of clinical presentation. There were no trends toward increased overall rates of diagnosis between 1982 and 1998 even though some of the 31 disorders were first recognized during these periods.

Between 1998 and 2002, when all infants were tested between 48 and 72 hours after birth, 57 infants were diagnosed with one of the 31 inborn errors or 15.7 diagnoses per 100,000 births. Of these, 48 infants were diagnosed by screening, while six were diagnosed clinically before or at the same time as the screening result became available, usually within 24 hours of testing. Two patients, siblings with ornithine transcarbamylase deficiency born to a mother [illegible] known risk, did not undergo screening. Seven [illegible]

whose diagnoses were made later on clinical grounds had negative results on newborn screening.

Although results showed an increase in the rate of diagnosis following the introduction mass spectrometry screening in newborns, most of the increase could be accounted for by the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency and to a lesser extent by the diagnosis of other disorders of fatty acid oxidation.

The authors calculated the cost of establishing a diagnosis. The incremental cost of the tandem mass spectrometry screening was $0.70 (USD) per newborn. The cost of confirmatory testing was $217 and the cost per relevant disorder detected was $3,939 if PKU was excluded or $2,519 if it was included. They concluded that their approach provides a rapid and inexpensive way to screen for a wide range of very rare metabolic diseases and that it identifies more cases than are diagnosed clinically. However they caution that it is not yet clear which patients identified through newborn screening would have become symptomatic if screening had not been performed.

Wilcken B et al. *New Eng J Med* 2003;348:2304-2312.

***Editor's Comment**: This paper brings to the fore the debate over the extent to which tandem mass spectrometry technology should be used to screen for a growing number of inborn errors of metabolism. As noted in a recent article by Marshall,[1] the debate pits parents and often physicians who advocate the application of this technology against ethicists with concerns over costs and public health officials with concerns over how the potentially large amount of genetic data will be managed. The Wilcken study demonstrates the successful implementation of the technology in a public health setting. It documents that the technology leads to an increased rate of diagnosis at low cost, especially for disorders of fatty acid oxidation, although acknowledges the possibility that some patients diagnosed as newborns may not have become symptomatic if screening had not been performed. Readers should note that metabolic screening by tandem mass spectrometry was highlighted by a recent lead article in GGH.[2] This article explains how technology works, provides guidelines for its use and describes its successful application in North Carolina. Together, these articles provide support for advocates of wider use of tandem mass spectrometry for newborn screening.*

References

1. Marshall E. *Science* 2001;294:2272-2274.
2. Millington D, Koeberl D. *GGH* 2003;19:32-38.

William A. Horton, MD

The Effect of Clitoral Surgery on Sexual Outcome in Individuals Who Have Intersex Conditions with Ambiguous Genitalia: A Cross-Sectional Study

It is estimated that intersex conditions occur in one per 2,000 live births. In the past, treatment had been based on the assumption that infants were gender neutral at birth, and that assignment of sex of rearing in early years which is reasonably compatible with the appearance of the external genitalia would provide a normal gender identity and partner orientation in adulthood. Subsequently, it has been recognized that there is a complex interaction between prenatal and postnatal factors that lead to the development of gender and sexual identity.

In the United States and in most western European societies, female rearing was most frequently recommended to parents whose infant had ambiguous genitalia. When the decision to raise the child as a female was made, surgery was usually undertaken to remove any ambiguity of the genitalia and to feminize the external appearance. This was done with the hope of a good psychosocial outcome.

Minto et al undertook a study involving individuals with several intersex conditions which included ambiguous genitalia, and who were living as adult females. Individuals were recruited from the Androgen Insensitivity Syndrome Support Group, the Adrenal Hyperplasia Network and the Intersex Clinic at University College in London Hospital.

Questionnaires were distributed and individuals could respond anonymously or identify themselves, in which case, their records would be examined with their permission. The self-administered questionnaires included the Golombok-Rust inventory of sexual satisfaction (GRISS) for women. Of the 39 patients included in this study, 11 had no clitoral surgery and 28 had had clitoral surgery. Almost all individuals who had undergone gonadectomy were taking hormone replacement therapy. Historical trends were noted in that most individuals seen before 1979 had undergone clitorectomy, while those operated on since 1980 usually underwent nerve-sparing clitoral reduction surgery. Many individuals also had vaginal reconstructive surgery.

The authors did multiple types of analysis of the data; however, the bottom line is that of the 39 participants, 13 individuals had never been sexually active and the 28 sexually active individuals had below normal scores in terms of sexual function. A low score on sensuality

was evident in the clitoral surgery group when compared to the non-surgical group. Both groups had difficulty with orgasm, which is relatively rare in a sexually healthy population. Of the 28 who had clitoral surgery, 18 found it impossible to have orgasm, compared with none among those who had not had clitoral surgery.

It was difficult to determine exactly why most of the study individuals were having difficulty with sexual function because only a questionnaire was used to obtain the data. There did not appear to be a difference among those patients recruited from the clinic versus those in support groups.

It would appear that genital surgery at a young age did not lead to satisfactory gender identity and sexual activity. However, it is not clear what the most appropriate approach should be. The authors encourage debate about the ethical issues, the development of reliable information, support of research in this area and how important it is to share this information with parents and patients who are considering clitoral surgery.

Minto CL et al. *Lancet* 2003;361:1252-1257.

***First Editor's Comment**: The outcomes of the management of intersex are not perfect. This study following up on previously treated individuals suggests that clitorectomy does not lead to sexual satisfaction, however, neither does clitoral reduction. Clearly, more research and discussion are needed in this area.*

Judith G. Hall, OC, MD

***Second Editor's Comment**: As the authors acknowledge, interpretation of their study is hampered by the small number of study subjects and the possibility that those electing to participate were among the more dissatisfied patients contacted initially. Quite interesting are the data that indicate that clitoromegaly itself is associated with sexual dysfunction. In addition to the concept that clitoral recession will permit the child to more readily accept her female sex assignment, the procedure is performed to ease parental acceptance of their newborn child. Those who have dealt on a personal and daily basis with parents of children with ambiguous genitalia know the need to assure and reassure parents is a paramount goal which is difficult to attain. Early clitoral recession by a skilled surgeon is most often recommended by this writer in those neonates with more severe degrees of genital ambiguity.*

Because of widespread neonatal screening for CAH, there is an increasing number of females with the most severe form of genital ambiguity known as Prader V or complete incorporation of the urethra into the phallus/clitoris. In the opinion of this writer and many others it is inappropriate to rear these genotypic and potentially fertile girls as males, thus necessitating genital surgery. Since both clitoromegaly and clitoral surgery impede sexual satisfaction, the challenge is to devise a corrective procedure that does not do so.

It would have been of interest to learn whether in those women with ambiguous genitalia who did not undergo clitoral surgery, clitoromegaly during childhood and young adulthood was a matter of significant concern. Counseling girls with ambiguous genitalia, whether operated upon or not, needs to begin in mid-childhood and to be conducted by individuals skilled in the management of this problem, as mentioned by Slijper in an excellent commentary regarding this article, in the same issue of Lancet (2003;361:1236-1237).

Minto's article also provides further support for the antenatal treatment with glucocorticoids of women bearing female CAH offspring at risk for development

Table

Sexual function of 28 participants, according to GRISS

	Subscale scores (%)					
	Clitoral surgery group (n=18)			No clitoral surgery group (n=10)		
	Normal*	Difficulties†	Severe difficulties‡	Normal*	Difficulties†	Severe difficulties‡
Frequency	28%	72%	33%	30%	70%	30%
Communication	28%	72%	17%	20%	80%	20%
Satisfaction	61%	39%	0%	80%	20%	0%
Avoidance	28%	72%	22%	20%	80%	10%
Sensuality	22%	78%	22%	80%	20%	10%
Vaginal penetration§	33%	67%	33%	33%	67%	22%
Orgasm	39%	61%	28%	60%	40%	0%

*Score of 1-4. †Score 5-9. ‡Score of 8 or 9. §Four individuals chose not to answer the question on vaginal penetration.

Adapted from Minto CL et al. *Lancet* 2003;361:1252-1257.

of ambiguous genitalia. It will be of great interest to assess the psychosexual development, orientation, and sexuality of these subjects as adult women. With the observations collected to date the impression is that they are normal little girls.

Allen W. Root, MD

***Third Editor's Comment**: The topic of intersex management, outcome, and research has received much attention in the past 2-3 years. The reader should be aware of publication of a collection of excellent papers presented in May 2002 at a conference entitled "Genetic and Hormonal Basis of Sexual Differentiation Disorders" (The Endocrinologist 2003;13:175-287) and of a "Summary of a Research Workshop on Intersex" held in sequence with the above conference (to be published in The Endocrinologist). Furthermore an excellent review entitled "Management of Children with Intersex Conditions: Psychological and Methodological Perspectives" bv S. Berenbaum was presented in GGH 19:1.*

Robert M. Blizzard, MD

Neonatal Exendin-4 Prevents the Development of Diabetes in the Intrauterine Growth Retarded Rat

Intrauterine growth retardation (IUGR) has been shown to be associated with significant adult morbidity, including insulin resistance, reduced pancreatic β-cell mass, and subsequent type 2 diabetes. Uteroplacental insufficiency, a cause of IUGR, limits the availability of substrates, growth factors, and hormones to the fetus. A rat model of IUGR can be induced with bilateral uterine artery ligation at 19 days of the 22 day gestation period. In rats during the newborn period there is extensive remodeling of the pancreas brought about by β-cell replication, neogenesis and apoptosis. A second wave of neogenesis occurs during weaning.

The incretin hormone glucagon-like polypeptide-1 (GLP-1) stimulates pancreatic neogenesis and increases β-cell mass. Therefore its administration to rat pups who have undergone 90% partial pancreatectomy results in an increase in both β-cell mass and improved glucose homeostasis. Exendin-4 is a long-acting GLP-1 which in addition to the aforementioned activities stimulates expression of Pancreatic Duodenal Homeobox (PDX) protein in the pancreas. PDX is critical for the early development of both the endocrine and exocrine pancreas and mediates glucose responsive stimulation of transcription of the insulin gene.

Stoffers and colleagues treated IUGR rat pups with exendin-4 during the early postnatal period to study its effects on the subsequent development of type 2 diabetes. Four groups of rat pups were studied: (1) control pups given vehicle injection, (2) control pups given exendin-4 injections, (3) IUGR pups given vehicle injections, and (4) IUGR pups given exendin-4 injections. Injections were administered on postnatal days 1 through 6. Glucose tolerance, β-cell mass, β-cell proliferation and PDX gene expression were measured at 14 days and 3 months of age. Glucose tolerance was also determined at 7 weeks and 8 months of age.

Exendin–4 decreased weights in both control and IUGR pups (Groups 2 and 4) at 2 weeks. This decrease persisted into adulthood (Table). At day 14, glucose tolerance in the IUGR pups treated with exendin-4 was similar to that in control animals. The treated animals remained euglycemic at 8 months. Vehicle–treated IUGR pups (Group 3) developed diabetes by 3 months and died by 8 months of age. Exendin-4 treated IUGR pups (Group 4) had normal β-cell mass comparable to that in Group 1 as the result of normalized replication rates. While Pdx-1 mRNA levels were reduced by 60% in IUGR rats not receiving exendin-4 at 14 days, those treated with exendin-4 had normal levels.

The authors state their major finding is that a short treatment with exendin-4 during the early newborn period prevents the development of diabetes in the IUGR rat. It is not clear whether this effect is through the stimulation of Pdx-1. However, the effect is independent of β-cell mass, since its effects were observed prior to any reduction in the IUGR pancreatic mass. They suggest that the permanent improvement in maintenance of β-cell mass by exendin-4 may mean that similar drugs could be effective in reducing the risk or preventing type 2 diabetes mellitus in individuals born with IUGR. The negative part of the study was the growth inhibiting effect of exendin-4.

Stoffers DA, et al. *Diabetes* 2003;52:734-740.

Table

Body weight at 2 weeks and 3 months

Treatment group	2 weeks (g) (n=9)	3 months (g) (n=7)
Control vehicle	27.7 ± 0.3	331.7 ± 7.0
Control Ex-4	22.2 ± 0.6*	305.3 ± 12.7*
IUGR Ex-4	13.8 ± 0.7†	311.0 ± 4.0†
IUGR vehicle	17.2 ± 0.7‡	351.7 ± 26.2‡

Data are means ± SE. *P < 0.05 control Ex-4 vs. control vehicle; †P < 0.05 IUGR Ex-4 vs. IUGR vehicle; ‡P < 0.05 control vehicle vs. IUGR vehicle.

Adapted from Stoffers DA, et al. *Diabetes* 2003;52:734-740.

Editor's Comment: *These fascinating data suggest that possibly there may be a treatment available in the future for the prevention of type 2 diabetes mellitus in IUGR individuals, if treated early in the neonatal period. Stoffers and colleagues have shown using an IUGR rat model that exendin-4 given for a short period of time postnatally can prevent glucose intolerance by restoring Pdx-1 function and normalizing β-cell proliferation rates. One cannot read this study without thinking about other morbidity associated with IUGR and how other treatments administered in the neonatal period might someday become available to treat those as well. The obvious example would be treatment given early to restore normal growth velocity. These authors have presented data that opens up a whole new world of possibilities.*

William L. Clarke, MD

Morbid Obesity and Mutations in Appetite Controlling Genes

It is known that the loss of function mutations of the melanocortin 4 receptor (MC4R) gene lead to severe obesity in humans and in mice. These genetic mutations disrupt the appetite control centers in the hypothalamus and lead to severe obesity. In the March 20, 2003 issue of the *New Eng J Med*, two papers were published which clarify the clinical syndrome resulting from the mutations in the appetite controlling MC4R gene.

In the first paper, Farooqi et al determined the nucleotide sequence of the MC4R gene, which is known to be a cause of a monogenic form of obesity. They studied 500 probands with *severe* obesity. In these families they examined the cosegregation of identified mutations, and in the subjects who were found to have MC4R deficiency they performed a metabolic-endocrine evaluation and characterized their clinical phenotype. The results were correlated with the signaling properties of mutant receptors. Twenty-nine probands (5.8%) had mutations in MC4R; 23 were heterozygous and 6 were homozygous. Mutation carriers were severely obese; their mean percentage of body fat was 43% of their body composition. Excess body weight gain was evident since the first year of life. They also presented increased lean body mass, increased linear growth, hyperphagia and severe hyperinsulinemia. The serum leptin and lipid levels, the metabolic rate, and thyroid, adrenal and reproduction function were normal. Homozygous individuals were more severely affected than the heterozygous ones. The subjects with mutations who retained some residual signaling capacity had a less severe phenotype than those with a totally absent signaling capacity. MC4R mutations resulted in a distinct obesity syndrome inherited in a co-dominant manner. The authors concluded that MC4R alterations play a key role in the development of a distinct form of severe obesity commencing in early childhood.

In the second paper, Branson et al studied the interactions of genetic and environmental factors which may have a bearing on the development of obesity in MC4R affected individuals. Four hundred sixty-nine severely obese white subjects with an average age of 42 years and with a mean body-mass index of 44, and 25 control subjects with normal weight and no history of obesity or dieting were included in this study. They sequenced (1) the complete MC4R coding region, (2) the proopiomelanocortin gene (POMC) region which encodes the α melanocyte-stimulating hormone (MSH), and (3) the binding domain of the leptin receptor (LEPR) gene. They also obtained detailed data concerning phenotypes, resting energy expenditure, diet-induced thermogenesis, serum leptin levels, and eating behaviors. Twenty-four of the 469 obese subjects (5.1%) and one of the 25 controls (4%) had MC4R mutations, including 5 novel variants. All mutation carriers reported binge-eating behavior, defined as repeated rapid consumption at least twice per week of an unusually large amount of food *in the absence of hunger*, causing the subject to feel embarrassed, depressed or guilty and out of control. This 100% prevalence of binge eating in MC4R mutation patients was compared with a 14% frequency of such behavior in obese subjects without genetic mutations. The prevalence of binge eating was similar among carriers of mutations in the LEPR as among that of non carriers. No mutations were found in the region of POMC encoding α MSH. The authors concluded that *binge eating* is a major phenotypic characteristic of subjects with a mutation in MC4R, a candidate gene for the control of eating behavior.

Farooqi IS et al. Clinical Spectrum of Obesity and Mutations in the Melanocortin 4 Receptor Gene. *New Eng J Med* 2003;348:1085-1095.

Branson R et al. Binge Eating as a Major Phenotype of Melancortin 4 Receptor Gene Mutations. *New Eng J Med* 2003;348:1096-1103.

Editor's Comment: *These two papers simultaneously published in the New Eng J Med are landmark studies. They contribute greatly to the understanding of the pathogenesis of obesity in humans. Farooqi and colleagues determined what proportion of obesity is attributed to a mutated gene of MC4R. They found that about 6% of severely obese individuals who had obesity since early childhood had these mutations. Thus patients carrying MC4R mutations consti[illegible]*

important subgroup of the severely overweight population. Given the high prevalence of observed MC4R deficiency, it appears that this condition represents the most common form of monogenic obesity in humans. Pediatricians and pediatric endocrinologists should be on the look out for this, especially in children who gain excess weight beginning in early childhood. Clinically, these patients differ from those with Prader Willi syndrome, who also have another form of monogenic severe obesity, by the normal stature and muscle development which are abnormal in Prader Willi syndrome.

The second study showed that overweight people who are binge eaters are more likely to harbor genetic mutations of MC4R than overweight people who constantly overeat. Until now, binge eating was considered a psychological phenomenon or disorder. For the first time a genetically driven characteristic was demonstrated. MC4R mutations appeared to disrupt brain signals governing satiety.

Both studies clearly document that there are severely obese individuals who overeat, not because of lack of will power, but because they have a genetically determined pathological syndrome.

However, these data also demonstrate that there are some individuals who have genetically determined mutations, yet are not obese. The reverse also occurs; specifically, binge eating behavior may occur and not be found to be associated with genetic mutations of MC4R. Thus, these two reports also support the thesis that the etiology of obesity is multifactorial, even in individuals who have a genetically determined alteration in the appetite control centers in the hypothalamus. In these patients, as well as in other obesities, excess energy intake over energy expenditures must occur for obesity to develop.

The reader is encouraged to review these two papers in detail, as well as to study the accompanying editorial by List and Habener[1] who clearly described the model of the homeostatic circuit regulating energy balance via the MC4 receptor. These authors point out that several hormones are known to influence the appetite control centers in the hypothalamus (Figure). MC4R deficiency is clearly implicated in the etiopathogenic mechanisms in some cases of severe obesity and binge eating, through short-circuiting the regulation of appetite in the hypothalamus. MC4R deficiency decreases the signals of anorixegenic pathways, such as CRH and TRH; and prevents the inhibition of orixegenic pathways, such as MSH and orexin. The result is increased food intake. The melanocortin agonist α MSH is a peptide that is produced by the POMC, and is an agonist of MC4R. On the other hand, leptin reduces food intake through stimulation of the expression of POMC and the production of MSH, while inhibiting MC4R antagonists such as the agouti-related protein.

The abnormal molecular physiology demonstrated in MC4R deficient patients constitutes an important discovery of a missing link between genes and behavior. However, there is a lot more to be uncovered before we fully understand satiety in individuals with MC4R gene mutations, as well as in other obesity syndromes, and in normal individuals.[2]

Fima Lifshitz, MD

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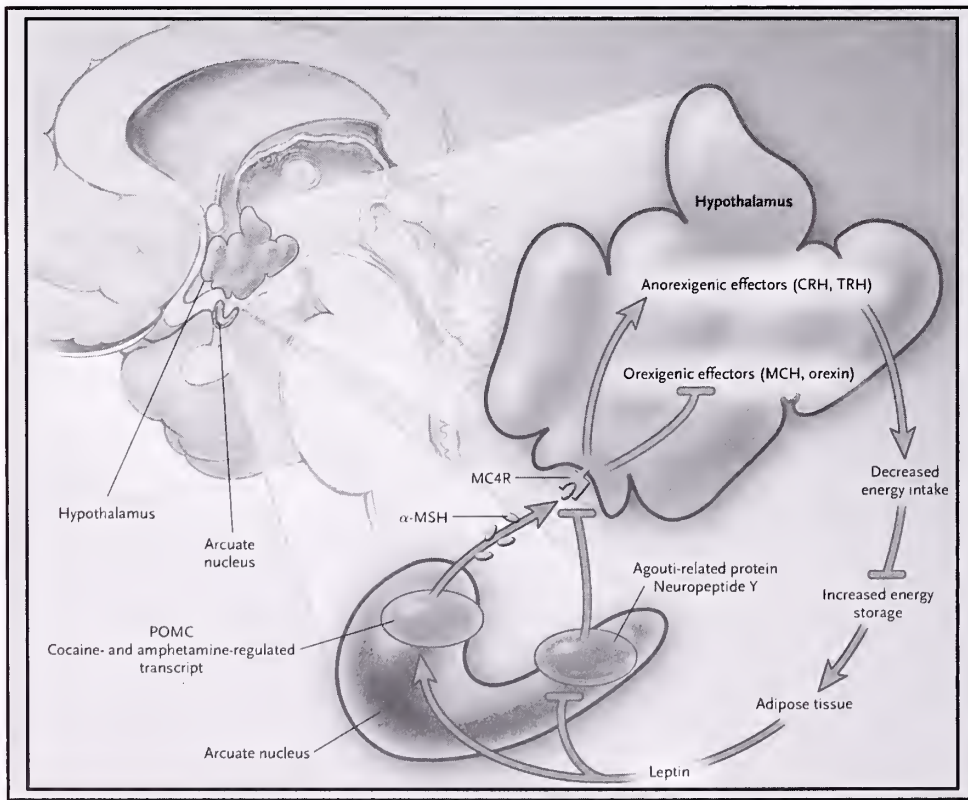
1. List JF, Habener JF. *New Eng J Med* 2003;348:1160-1163.
2. Gotoda T. *N Eng J Med* 2003;349:606-609.



Figure

Model of Homeostatic Circuit Regulating Energy Balance through the Melanocortin 4 Receptor (MC4R). Increased adiposity leads to increased leptin production in fat tissue. Leptin stimulates neurons in the arcuate nucleus of the hypothalamus that coexpress the anorexigenic hormones a melanocyte-stimulating hormone (α-MSH, a cleavage product of proopiomelanocortin [POMC]) and cocaine- and amphetamine-regulated transcript. Leptin also inhibits neurons in the arcuate nucleus that coexpress the orexigenic hormones agouti-related protein and neuropeptide Y. The neurons in the arcuate nucleus project to other regions of the hypothalamus (including the paraventricular nucleus and the lateral hypothalamic area–parafornical area), where α-MSH binds to its receptor, MC4R, resulting in an up-regulation of anorexigenic effectors such as corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) and a down-regulation of orexigenic effectors such as melanin-concentrating hormone (MCH) and orexin. Agouti-related protein acts as an antagonist of MC4R.

Hypogonadism and Pubertal Development in Prader-Willi Syndrome

Genital abnormalities are common in Prader-Willi Syndrome (PWS) and are one of the eight major clinical criteria for diagnosis. Previous reports of the type and frequencies of these abnormalities were not necessarily from individuals with genetically confirmed PWS. Crino and associates report data from patients evaluated by the Genetic Obesity Study Group of the Italian Society of Pediatric Endocrinology and Diabetology. Eighty-four patients (42 males), mean age 15.8±8.2 years were studied. Sixty-three percent were over 14-years-old. All satisfied the Holm and Cassidy clinical criteria for the diagnosis of PWS and the methylation test was positive in all subjects. Microdeletion of chromosome 15(15q12-13) was demonstrated in 66%, while uniparental disomy or an imprinting defect was suspected in the others.

All males showed cryptorchidism (86% bilateral). Small testes and scrotal hypoplasia were observed in 76% and 69%, respectively. Micropenis was seen in 36%. Twenty-two of 29 males had spontaneous onset of puberty at 14.0±3.2 years but it was incomplete in all cases. Specifically, pubertal changes past Tanner 2-3 genital stages were rarely observed.

In females there was hypoplasia of the labia minora and/or of the clitoris in 71% and 69% of cases. Thirty-four of 39 females had spontaneous onset of puberty at 12.6±2.7 years, with very slow progression. Menarche occurred at a mean age of 17.3±5.2 years in 44% of cases over 14 years of age. Primary amenorrhea was diagnosed in 56%. Menstrual cycles were seldom regular and secondary amenorrhea occurred in 33% who had spontaneous menarche. Of note, premature pubarche occurred in 12 subjects (6 males) and true precocious puberty in 3. It is suggested that premature pubarche might have been related to obesity. Genital and pubertal abnormalities were evenly distributed among subjects with microdeletion and UPD-imprinting defects. Treatment of various types for hypogonadism was discussed, including the use of dihydrotestosterone transdermally. However, no systematic trials on treatment with sex hormone treatment in adolescents or adults are available.

Crino A et al. *Eur J Pediatr* 2003;162:327-333.

***Editor's Comment**: This paper provides interesting information concerning genital abnormalities in individuals diagnosed with PWS, confirmed with genetic testing. The large number of subjects in this descriptive study and the careful presentation of the findings should assist all who work with these patients and who must counsel them and their families in regard to expectations for pubertal development and fertility. It is interesting that sexual precocity was observed at a frequency that should be considered high in this group. This suggests that examination of the genitalia should be performed at each clinical visit. Whether or not current treatment with exogenous GH, which has been shown to significantly alter body composition in PWS, will affect pubertal development remains to be shown.*

William L. Clarke, MD

Growth and the Tyrosine Kinome

Tyrosine kinases (TKs) add phosphate moieties to tyrosine residues on proteins that typically serve as docking sites to recruit other molecules that bind and propagate signals. As such, they function as central regulators of signaling pathways that control transcription, cell cycle progression, differentiation, apoptosis and other processes that are highly relevant to growth of cells and tissues. Given this central position in regulation of growth, Bardelli et al raised the question: why have mutations in TK genes been found in only a small number of instances including certain human cancers? They speculated that mutations do exist, but have yet to be detected because the vast number of TK genes is only now becoming apparent as the human genome project unfolds. To test this idea, they took advantage of high-throughput sequencing and bioinformatics from the human genome project to search for TK mutations in a select group of cancers, colorectal cancers.

A recent analysis organized the protein kinase complement of the human genome (the "kinome") into a dendrogram containing nine broad groups or branches of genes. Bardelli et al selected one major branch, which contained three groups including 90 TK genes, 43 TK-like genes and 5 receptor guanylate cyclase genes. Mutation analysis of 813 exons from the genomic database carried out on DNA from 35 colorectal cancer cell lines yielded 14 mutations. Further analysis of DNA from 147 tumors identified 46 novel mutations in 14 genes. All of the mutations were somatic in nature based on comparison of DNA from tumor to matched normal tissues.

The authors suggested that mutations found in seven genes, which were detected in more than one tumor

were functional rather than coincidental. Based on the specific locations of the mutations, they further suggested that many of the mutations were activating in nature, i.e., they resided in key regions of the TK, such as the autoinhibitory activation loop. The authors concluded that at least 30% of colorectal cancers contain at least one mutation in the tyrosine kinome. They emphasized that an important reason to study TK genes is that they provide attractive targets for therapeutic intervention for growth disorders, noting the convincing success of targeting BCR-ABL tyrosine kinase in leukemia (Druker BJ. *Cancer Cell* 2002;1:31).

Bardelli A et al. Mutation Analysis of the Tyrosine Kinome in Colorectal Cancers. *Science* 2003;300:949.

***Editor's Comment**: While this paper specifically addresses cancer, it does not take too much imagination to see its potential relevance to growth of other tissues, such as the skeleton. Indeed, achondroplasia is due to activating mutations of the FGFR3 tyrosine kinase. Given the scope of regulation necessary to orchestrate and coordinate events in a growing bone, it seems highly probable that there are other members, perhaps many, of the tyrosine kinome involved. Accordingly, mutations of these as of yet undefined TKs may underlie disorders of skeletal growth. Considering the remarkable success of Gleevec in treating chronic myelogenous leukemia by inhibiting the BCR-ABL TK, it is not inconceivable to dream of using pharmacologic manipulation of growth-plate TKs to therapeutically manage certain bone growth disturbances in the future.*

William A. Horton, MD

What do Craniosynostosis and Kallmann Syndrome Have in Common? *FGFR1*

Kallman syndrome is characterized by loss of the sense of smell, anosmia and hypogonadotropic hypogonadism. The anosmia results from absence or hypoplasia of the olfactory bulbs and tracts. The hypogonadism is due to a deficiency of GnRH, probably the result of failure of GnRH-synthesizing neurons to migrate from the olfactory epithelium to the forebrain along the olfactory nerve pathway. Kallmann syndrome occurs mainly in males and most often is inherited in an X-linked recessive fashion; the gene responsible for this form has been identified, *KAL1*. However, there are instances, such as failure to detect a *KAL1* mutation, that suggest an autosomal form of Kallmann syndrome.

Through segregation analysis of polymorphic markers and FISH chromosomal analysis, Dodé et al identified two *de novo* deletions of about 11 Mb at chromosome 8p11.2-p12 in two individuals affected by different contiguous gene syndromes that included Kallmann syndrome. The overlapping region of about 540 kb contained three genes, one of which, *FGFR1* (fibroblast growth factor receptor 1) was considered a strong candidate for causing Kallmann syndrome because of its known interaction with the *KAL1* gene product, anosmin-1. Southern blot analysis of 43 individuals with familial or sporadic Kallmann syndrome failed to detect additional deletions of *FGFR1*. However, sequencing of *FGFR1* in 129 unrelated patients with Kallmann syndrome revealed heterozygous mutations in four familial and eight sporadic cases. The mutations, including nonsense, frameshift and splice-site mutations, predicted loss of *FGFR1* function.

These observations suggest that Kallmann syndrome can result from haploinsufficiency or reduced dosage for *FGFR1*. The authors point out that anosmin-1 binds to heparin sulfate proteoglycans which are required for FGF ligands to bind to FGF receptors and that *KAL1* and *FGFR1* are expressed in many of the same areas in the embryo including the region of olfactory bulb development. They offer a possible explanation for the higher prevalence of Kallmann syndrome in males even in families with autosomal inheritance which is based on the assumption that the local concentration of anosmin-1 is important to FGF signaling, and the observation that *KAL1* partially escapes X-inactivation. Accordingly, females with two *KAL1* alleles synthesize higher amounts of anosmin-1 than do males with a single *KAL1* allele. The authors propose that this may be enough in some cases to maintain FGF signaling above a critical threshold with regard to *FGFR1* signaling in the context of olfactory bulb and tract development.

Dodé C, et al. Loss-of-function Mutations in *FGFR1* Cause Autosomal Dominant Kallmann Syndrome. *Nat Genet* 2003;33:1-3.

***First Editor's Comment**: FGFR1 joins a small group of genes for which both gain and loss of function mutations are known and associated with disease. It is not surprising that gain and loss of function mutations lead to quite different clinical consequences. Gain of FGFR1 function causes craniosynostosis, especially Pfeiffer syndrome, while loss of FGFR1 function results in Kallmann syndrome. Thus, these two syndromes are technically allelic disorders. One wonders how common this phenomenon actually is. Indeed, those of us with interest in FGFR3 have pondered if some individuals with tall stature have loss of function mutations of this gene in contrast to the gain of FGFR3 mutations that cause achondroplasia.*

The paper also illustrates the importance of gene dosage. In some instances, the precise dosage of a gene or its product does not seem to matter so much. Examples include, metabolic disorders in which half the normal amount of enzyme is more than enough to prevent disease and mutations of structural proteins, where inclusion of variable amounts of abnormal gene product can disrupt the formation of multimeric molecules containing the products of both mutant and normal alleles. When mutations involve regulation, such as mutations that affect signaling or formation of transcription factor complexes, small differences may have large effects on the outcome of the regulated events, especially if they involve thresholds as proposed for FGFR1 signaling in this report.

William A. Horton, MD

***Second Editor's Comment**: The authors have identified a second gene involved in the pathogenesis of Kallmann syndrome. The large number of subjects with Kallmann syndrome (N=116) in this study in whom mutations in neither KAL1 or FGFR1 were found indicates that there are (many) more genetic mutation which lead to this disorder. Search for involved genes might be directed toward those that encode products known to be important in neural cell migration and upon the intracellular proteins that are phosphorylated and the downstream genes whose transcription is regulated by FGFR1. It is interesting (curious?) that gain-of-function mutations of FGFR1 are associated with the Pfeiffer syndrome of craniosynostosis, but that inactivating mutations of this gene have not been linked to delayed closure of cranial sutures.*

Allen W. Root, MD

Clinical, Autoimmune, and HLA Characteristics of Children Diagnosed With Type 1 Diabetes Before 5 Years of Age

Little is known about auxologic, autoimmune, and HLA characteristics specific to children with early-onset diabetes (EOD). In this paper 40 children with a mean CA of 2.6 years who developed diabetes mellitus before 5 years of age were studied. These patients were compared with a matched subgroup of children of mean age of 9.9 years, therefore, with later onset diabetes mellitus (LOD). Auxologic data and antibody radioimmunoassay data from medical records were retrospectively analyzed. HLA diabetes related class II alleles were typed and evaluated for comparison between "whites" and "Hispanics" . The frequencies of glutamic acid decarboxylase (GAD) and islet cell antibodies (ICA) were significantly lower in the EOD group than in the group developing diabetes at an older age. No significant differences were detectable for insulin auto-antibodies (IAA), thyroid peroxidase, and thyroglobulin antibodies. None of the patients of the EOD group developed hypothyroidism, whereas 20% of the LOD patients did. There was a negative correlation between GAD antibodies and the predisposing haplotype DR3/DQ2. None of the EOD patients had either of the protective alleles (DRB1*1501 or DQB1*0602) for diabetes. There were significant differences in the frequencies of some diabetes related HLA alleles between EOD patients and a large non-age stratified type 1 diabetes group. The pertinent clinical information, frequency of autosomal markers and HLA data among ethnic groups are below (Tables 1-3). The authors concluded that children with EOD have different diabetes related autoimmune and genetic characteristics from those diagnosed later on in life.

Hathout EH et al. *Pediatrics* 2003;111: 860-863.

***Editor's Comment:** Very young children with diabetes mellitus are known to have a more severe course than those diagnosed later in life. The difficulties in the control*

Table 1
Clinical Information on Study Children With Type 1 Diabetes

	Early-Onset Group (Diagnosis Age <5 Years)	Late-Onset Group (Diagnosis Age >5 Years)	*P* Value
Concomitant illness at diagnosis	72.73%	31.80%	<.01
Documented hon eymoon period	16.70%	42.10%	.24
DKA at diagnosis	80.80%	36.40%	<.01
Hemoglobin A1C at diagnosis	10.33 ± 2.20	11.27 ± 2.57	.21
No. of ICU days at diagnosis	1.82 ± 2.09	.53 ± .70	.07

DKA indicates diabetic ketoacidosis; ICU, intensive care unit.

Table 2
Frequency of Autoimmune Markers in Study Children With Type 1 Diabetes

Marker	Early-Onset Group (Diagnosis Age <5 Years; %)	Late-Onset Group (Diagnosis Age >5 Years; %)	P Value
TpoA	6	9	1.00
TGA	9	11	1.00
IAA	50	65	.43
GAD	32	77	<.01
ICA	29	68	<.01

TpoA indicates thyroid peroxidase antibody; TGA, thyroglobulin antibody

Table 3
HLA Data in the 2 Major Ethnic Subgroups of Study Children With Onset of Type 1 Diabetes Before 5 Years of Age

HLA Allele(s)	Percentage of Whites With Allele(s) (%)	Percentage of Hispanics With Allele(s) (%)
DRB1*0401-DQA1*03-DQB1*0302	70.6	.0
DRB1*0402-DQA1*03-DQB1*0302	.0	92.9
DRB1 0401	35.3	.0
DRB1 0405	.0	21.4

Tables 1-3 are adapted from Hathout EH et al. *Pediatrics* 2003;111: 860-863.

of the disease among the younger patients may account for more frequent and more severe complications of the disease occurring earlier in life. However, the data in this paper are suggestive that there are autoimmune and genetic differences among type 1 diabetic patients according to age (early vs late onset), and these may account for the differences in the control and the outcome of the disease. Chromosomal abnormalities (parental isodisomy of chromosome 6) also have been described among patients with the transient form of neonatal diabetes.[1] Studies like these suggest that EOD probably is not classic type 1 diabetes mellitus, and thus may require unique approaches for prevention and therapy.

Fima Lifshitz, MD

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1. Metz C et al. *J Pediatr* 2002;141:483-489.

The Thyrotropin Receptor Autoantigen in Graves Disease is the Culprit as well as the Victim

The thyrotropin (TSH) receptor (TSHR) is the only 7-transmembrane G-protein coupled receptor (GPCR) for glycosylated hormones that undergoes cleavage after its primary formation; the amino terminal extracellular domain is cleaved at/near amino acid 289 (*subunit* A) leaving a short residual extracellular amino acid sequence, the 7 transmembrane domains and extracellular and intracellular connecting loops, and the intracellular carboxyl terminal domain (*subunit* B). Subunit A then circulates and can serve as an immunogen. The role of *subunit* A of the TSHR in the pathogenesis of autoimmune hyperthyroidism and the development of TSHR stimulating immunoglobulin (TSIg) was examined by the present investigators. They constructed within adenovirus cDNA transcripts of the amino terminal 289 amino acid sequence (*subunit* A), the wild-type (wt) *TSHR* from which amino acids 317-366 had been removed rendering the truncated TSHR resistant to cleavage, and the intact wt *TSHR*.

Adenoviruses expressing different forms of the TSHR were then administered to female mice who subsequently developed abnormalities of thyroid function and antibodies of variable biologic activity in response to these proteins. In animals receiving TSHR 1-289, clinical, biochemical, and thyroid histologic evidence (thyromegaly, hyperthyroxinemia, and follicular hyperplasia) of thyrotoxicosis developed. These animals also developed TSIg (assessed by increase in cyclic AMP formation in CHO cells expressing TSHR). In only a few mice receiving cleavage resistant TSHR or wt

TSHR were serum thyroxine levels increased and thyroid follicular hyperplasia present. In contrast, all mice, regardless of the form of TSHR received, developed high but approximately equal titers of immunoglobulins that bound to TSHR or inhibited radiolabeled TSH from binding to TSHR. TSIg did not develop in animals receiving cleavage resistant TSHR, but did appear in 30% of those injected with wt TSHR. Higher titers of thyroid blocking antibodies (assessed by their effect on TSH mediated increase in cyclic AMP generation in CHO cells expressing TSHR) were present in mice receiving the cleavage resistant form of the TSHR than in those receiving TSHR 1-289. The authors conclude that it is the extracellular segment of the TSH receptor that is ordinarily shed that serves as the immunogen for the development of TSIg in this experimental model of hyperthyroidism (and by analogy in patients with Graves disease).

Chen C-R, et al. *J Clin Invest* 2003;111:1897-1904.

***First Editor's Comment**: This extremely interesting manuscript provides significant insight into the pathogenesis not only of thyrotoxicosis, but of autoimmune thyroid disease itself. Thus, when the ectodomain of the TSHR is cleaved, it provokes the production of TSHR stimulating immunoglobulins (as well as low titers blocking antibodies) in genetically susceptible individuals. In other at-risk patients, the intact TSHR (or perhaps other sequences or epitopes of the TSHR) or TSH itself, serves as the immunogen for development of TSHR function-blocking antibodies. Other components of the thyroid gland serve as immunogens for antibodies that are injurious to the thyroid cell. A human monoclonal antibody has been recently isolated from a patient with Graves disease, but the epitope of the TSHR to which it is directed has not been identified to date.[1,2] It would be of interest if it were directed to the ectodomain of the human TSHR.*

While a number of tyrosine kinase receptors shed their extracellular domains (growth hormone binding protein, prolactin binding protein, many cytokines), it is apparently unusual for G-protein coupled receptors to do so. This is an area that merits further examination.

Allen W. Root, MD

***Second Editor's Comment**: In Dr. Root's editorial comment, he refers to the recent identification of a monoclonal antibody that stimulates the TSH receptor in the thyroid cell to release thyroxin.[1,2] This also was no small accomplishment in helping us understand Graves' disease more fully. As pointed out by Dayan, who states:*

"So, is the final proof of the existence of thyroid-stimulating immunoglobulin after a journey of 47 years of anything more than academic interest? Almost certainly the answer is "yes." First, this finding might lead to a new generation of assays for thyroid-stimulating immunoglobulin in which competition for labeled TSH is replaced by competition for specific monoclonal antibodies. If a sensitive assay can be developed, it should have close to 100% specificity for Grave's disease and replace all other antibody tests, such as antithyroid peroxidase and antithyroglobulin, in this condition. Second, it should finally allow us to understand how such antibodies, even in the monomeric Fab form, can activate the TSH receptor. Such understanding of the biology of glycoprotein-hormone receptors may lead to new small-molecule agonists and antagonists not only for thyroid disease but also for hypogonadism and infertility (via the closely related receptors for luteinising and follicle-stimulating hormones). And it may prove possible to clone a potent human TSH-receptor-blocking antibody which might provide a rapid initial treatment for thyrotoxicosis. Third, the finding may lead to a better understanding of the pathogenesis of Graves' disease. How is it that the spontaneous development of such agonist antibodies, unique in autoimmune diseases, occurs so frequently (almost 1 in 100 of the population)? Does the agonist activity itself, once it appears, promote autoimmunity in a positive feedback loop? Most intriguingly, cloning of agonist TSH-receptor autoantibodies might reveal antibodies that contribute to thyroid eye-disease, the most mysterious manifestation of Graves' disease, and perhaps lead to inhibitors for these antibodies. And finally, agonist antibodies may prove a useful therapeutic agent in their own right, such as to enhance iodine-131 uptake in thyroid cancers. Many of the holy grails of biological science, from the structure of DNA to the nature of the T-cell antigen receptor, have been found. Thankfully, once in hand, they change into pointers to the many more waiting to be discovered."

The findings of Chen and those of Sanders et al are linked closely and the almost simultaneous reporting of these factors which are linked should permit a logarithmic advance in our understanding of how antibodies and receptor structure and function can relate and, consequently, provide better therapy of immunological diseases.

Robert M. Blizzard, MD

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1. Sanders J, et al. *Lancet* 2003;362:126-128.
2. Dayan CM. *Lancet* 2003;362:92-93.

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